

Figure 1

00000707 054304

00000727 001304

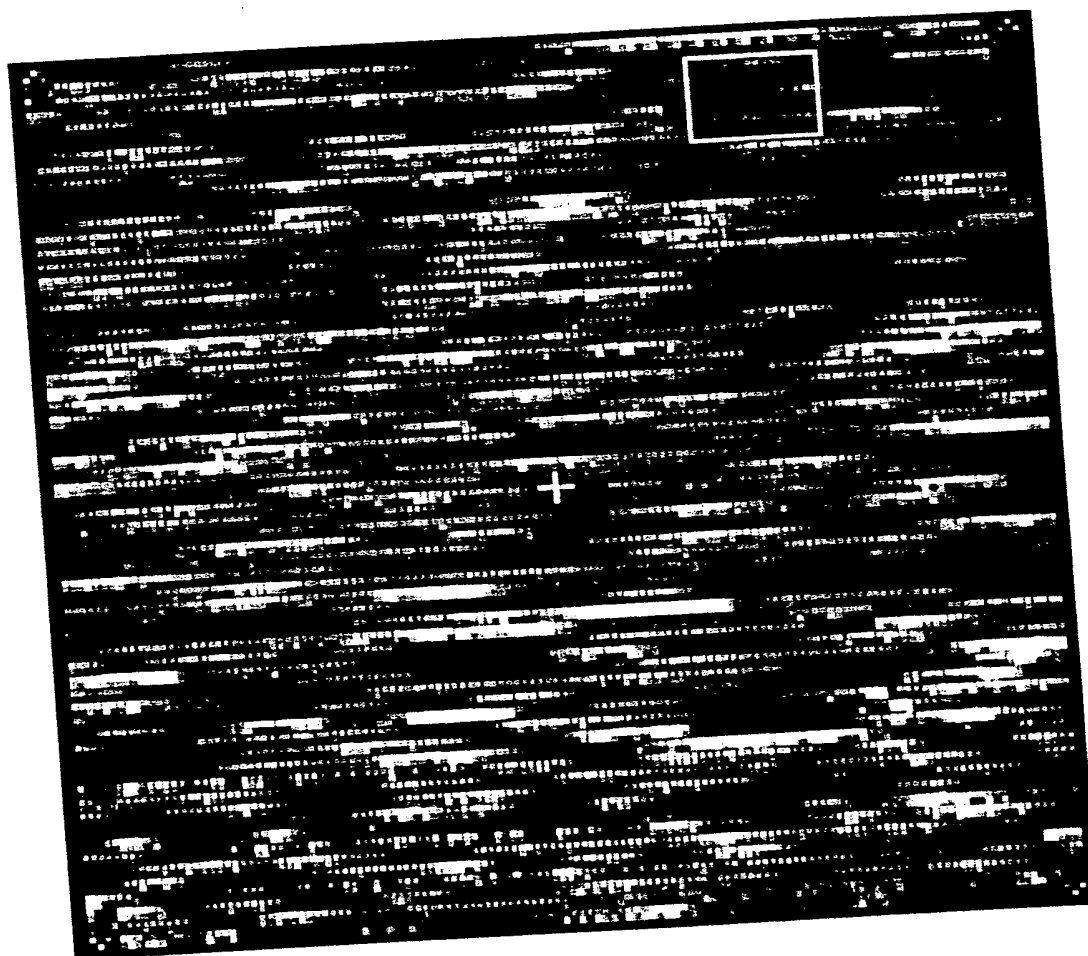


Figure 2a

Figure 2b

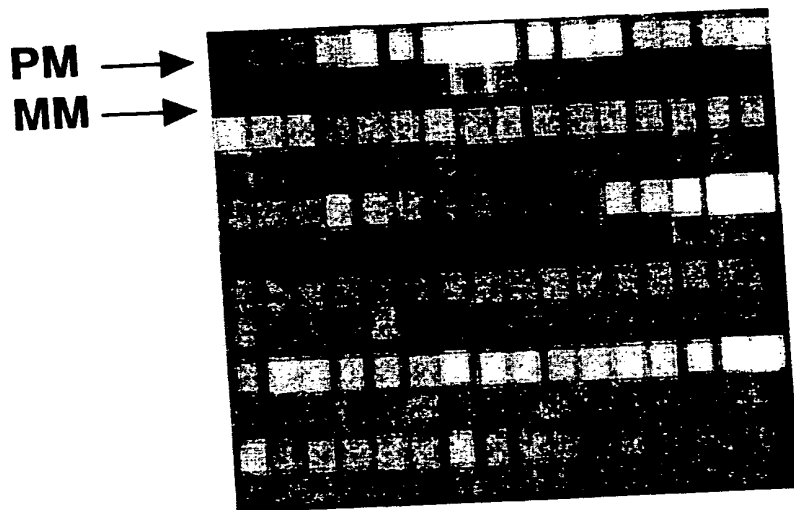


Figure 2c



00000727 001304

## Hybridization Signal vs Target Concentration

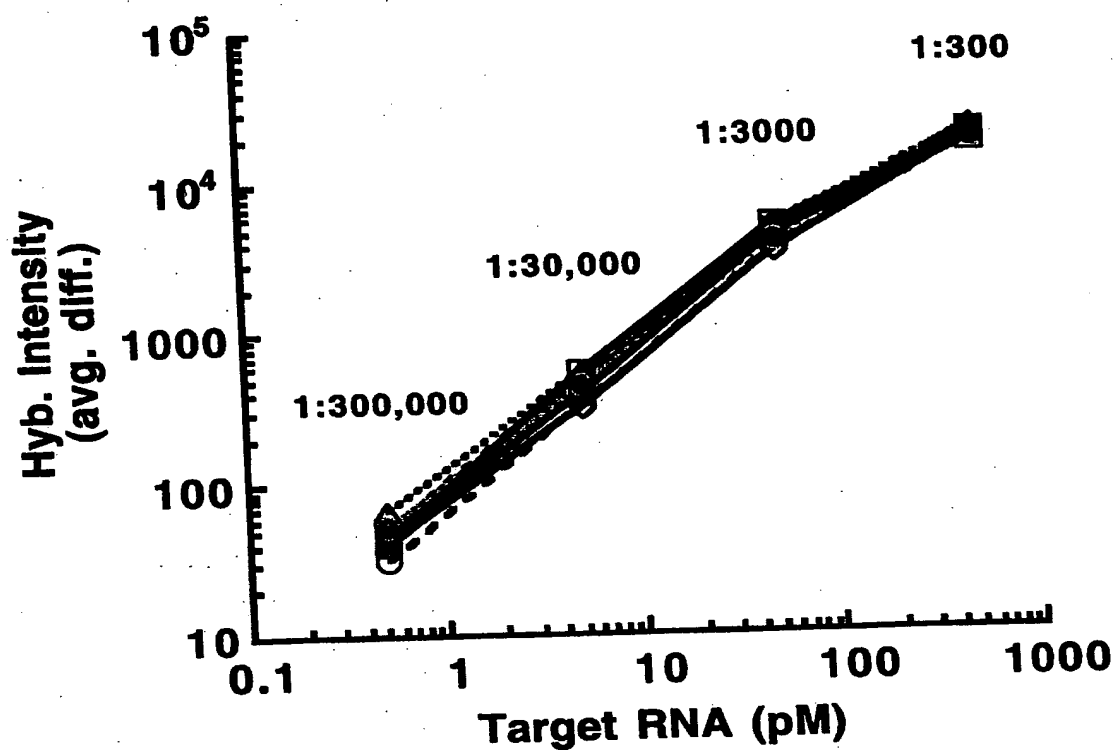


Figure 3

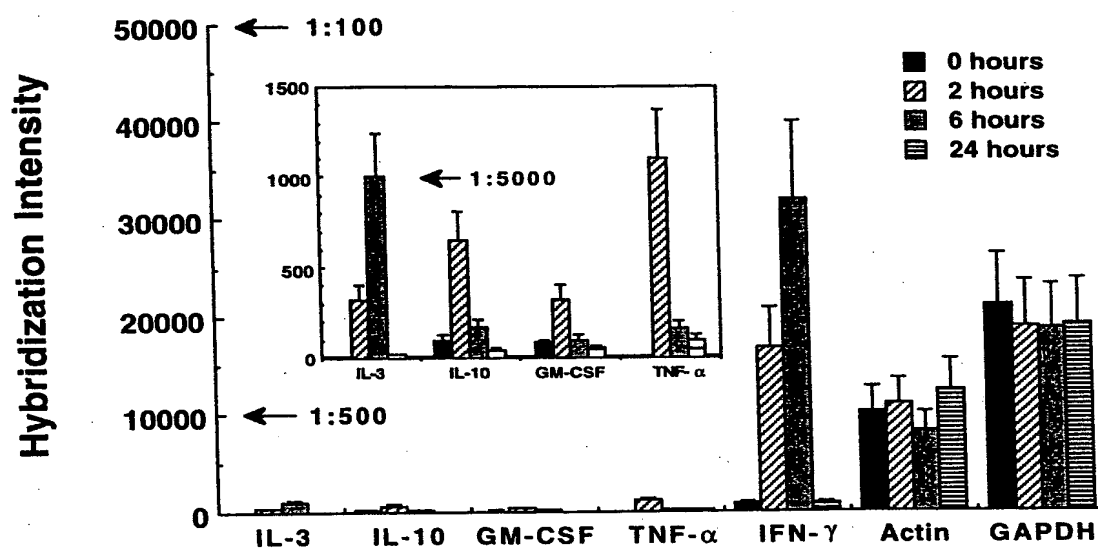


Figure 4

6/47

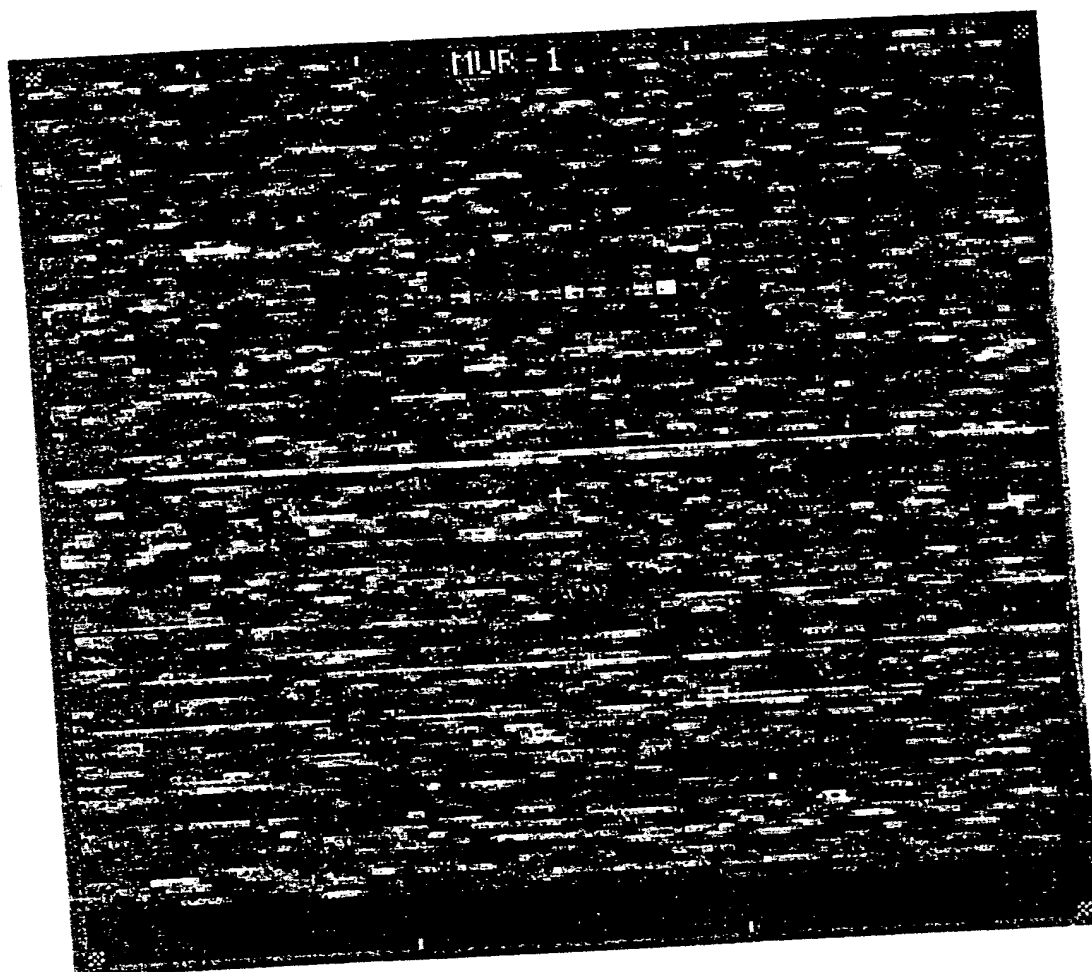


Figure 5

0000727 001304

7/47

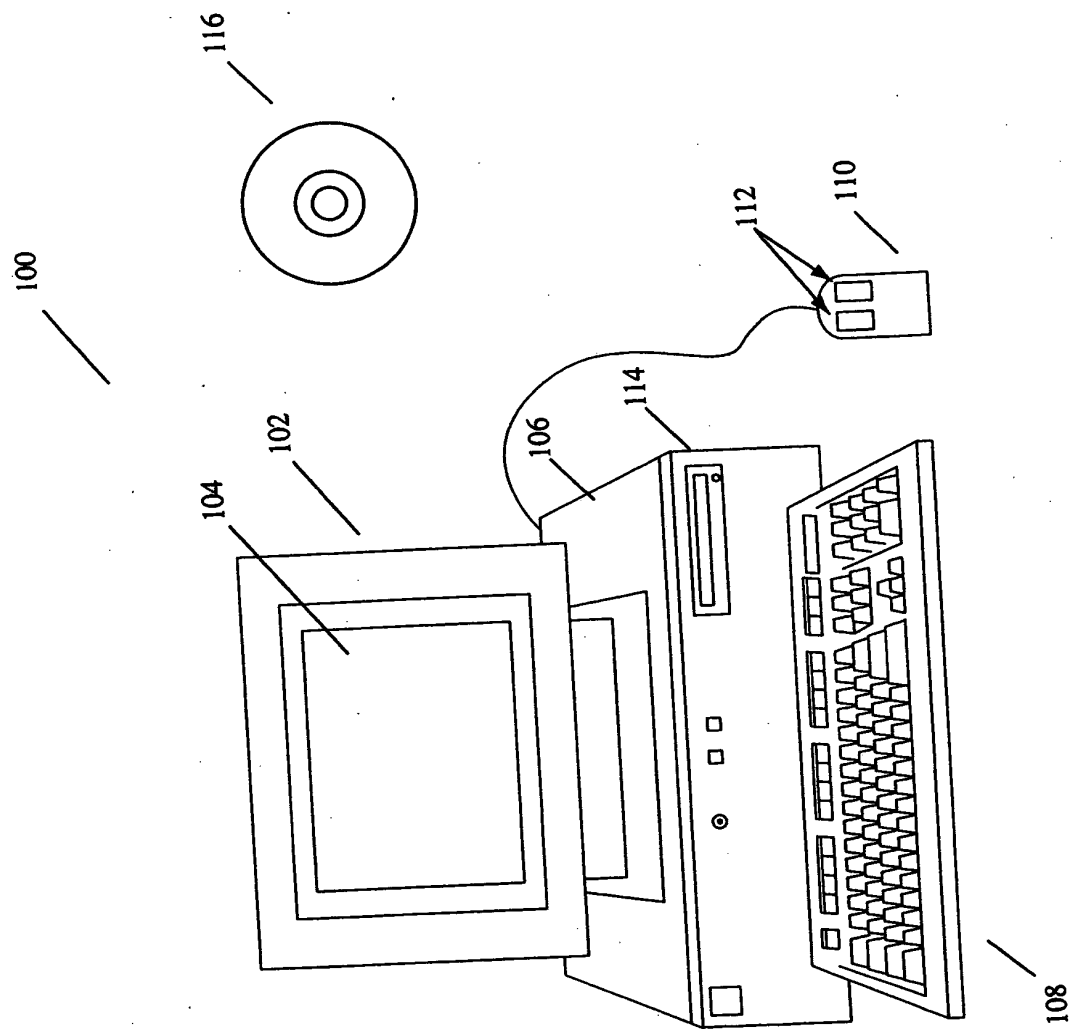


Figure 6

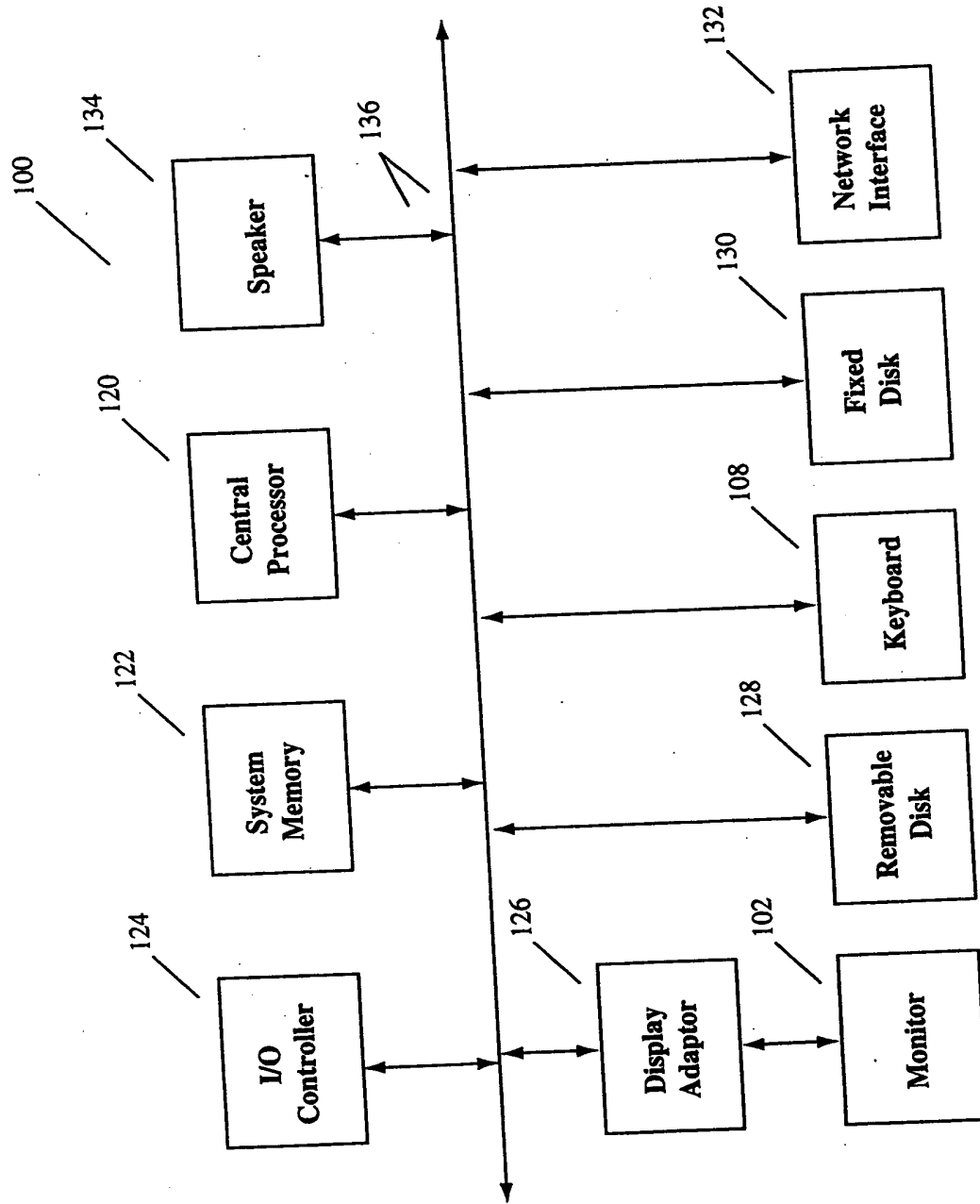


Figure 7



102790 12200000

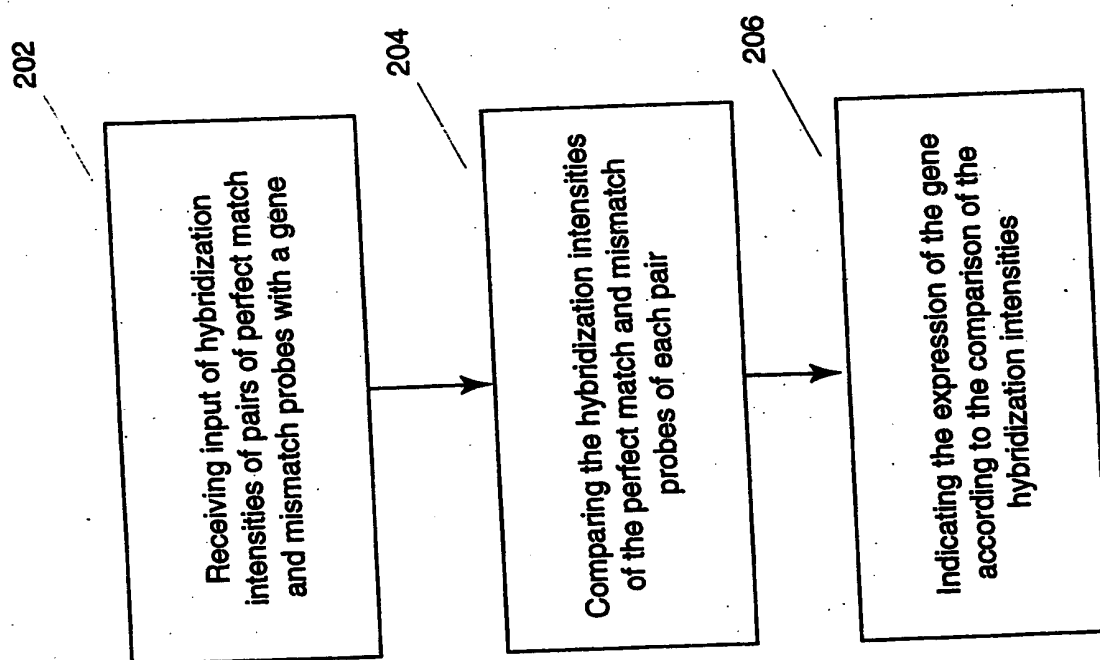


Figure 8

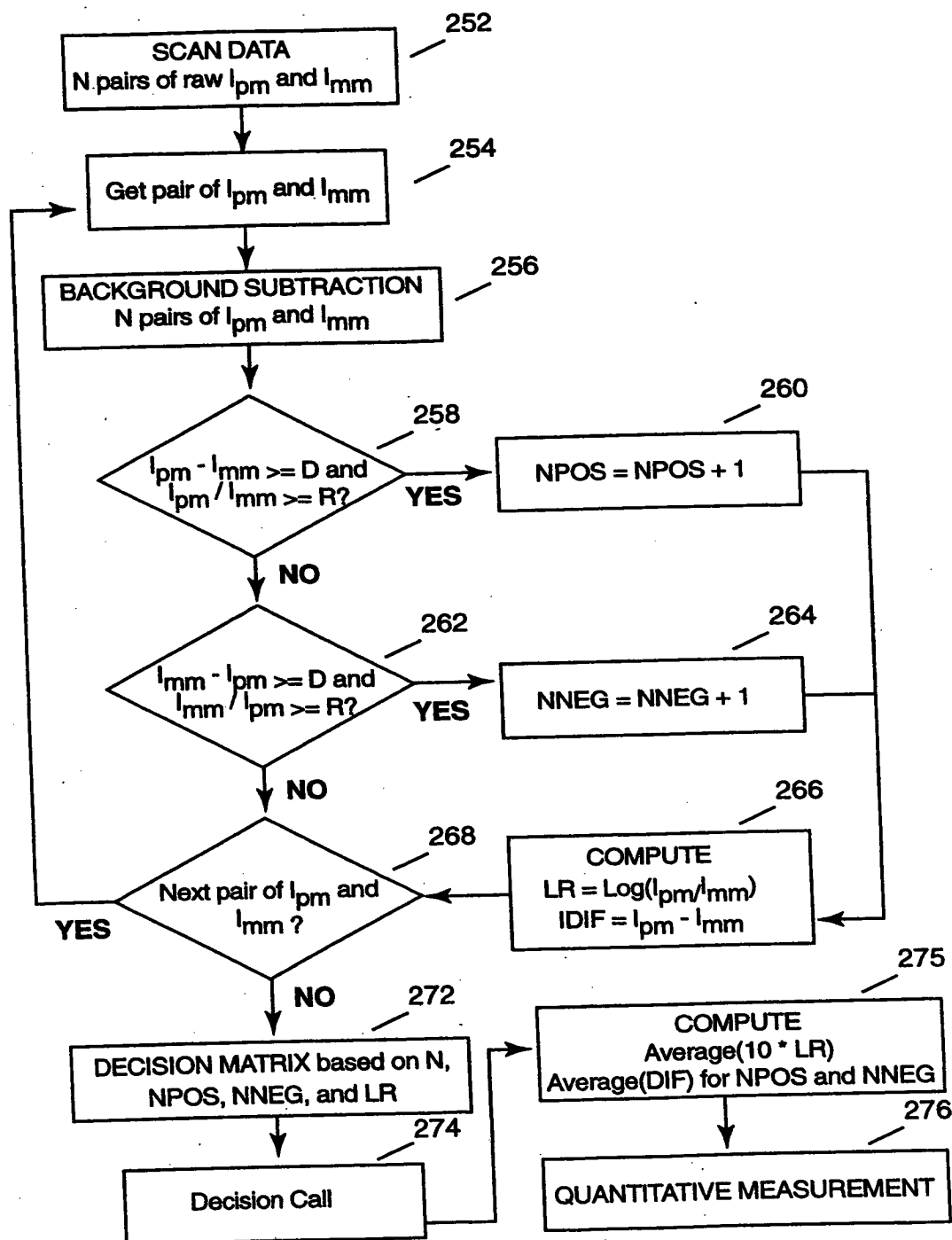


Figure 9

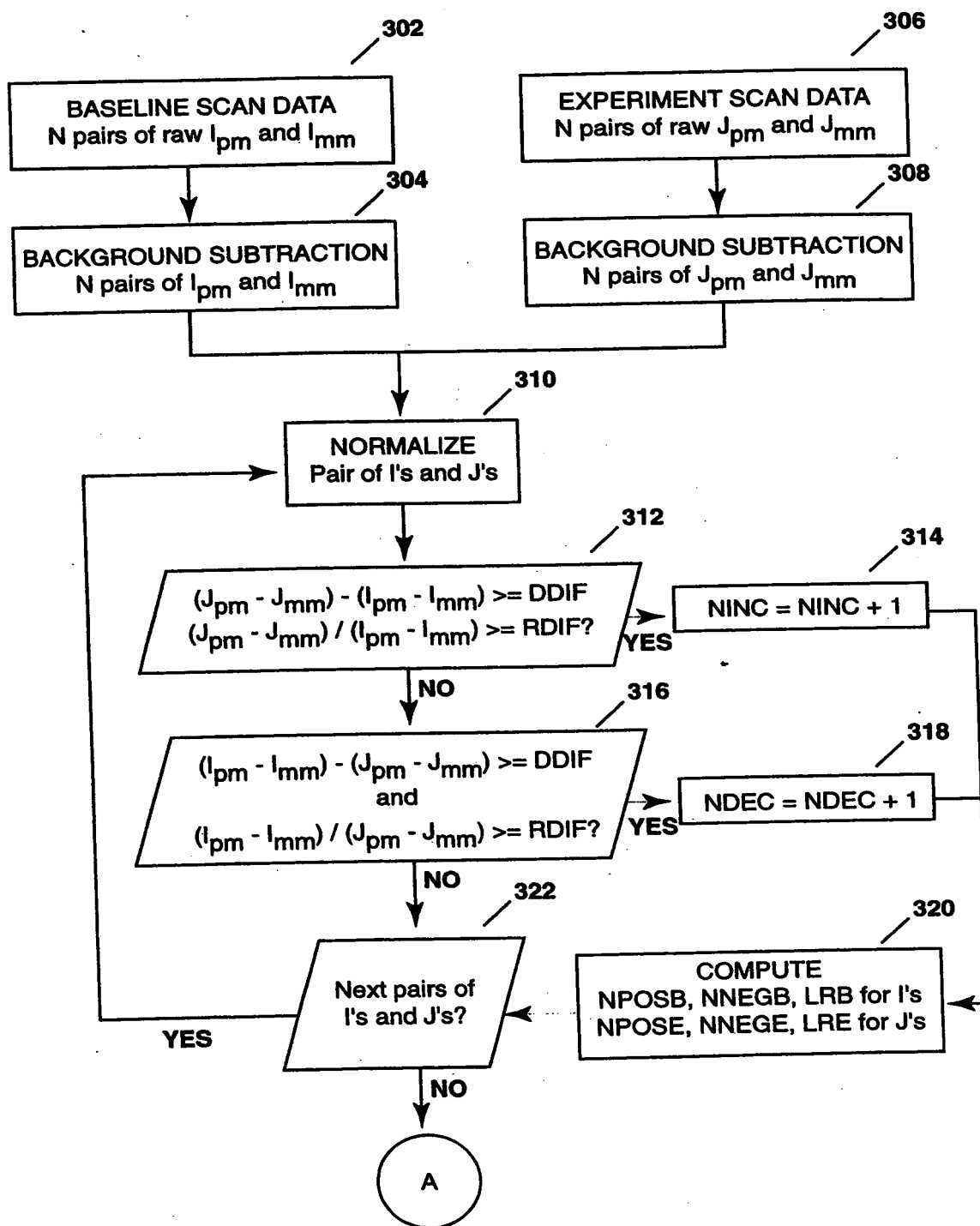


Figure 10a

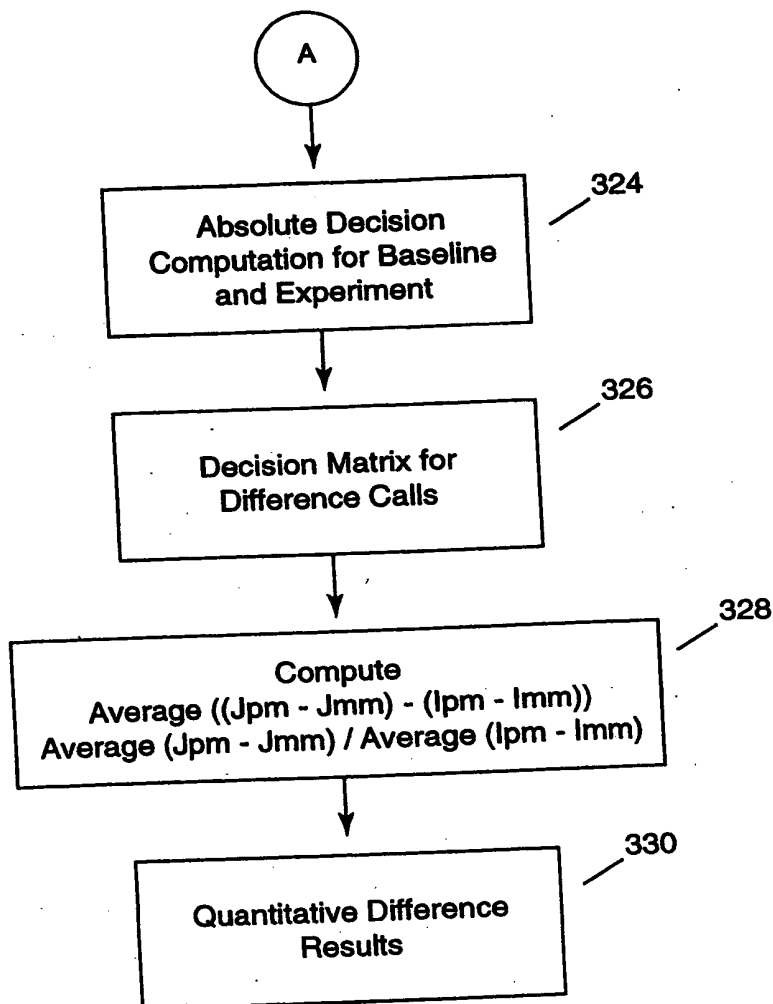


Figure 10b

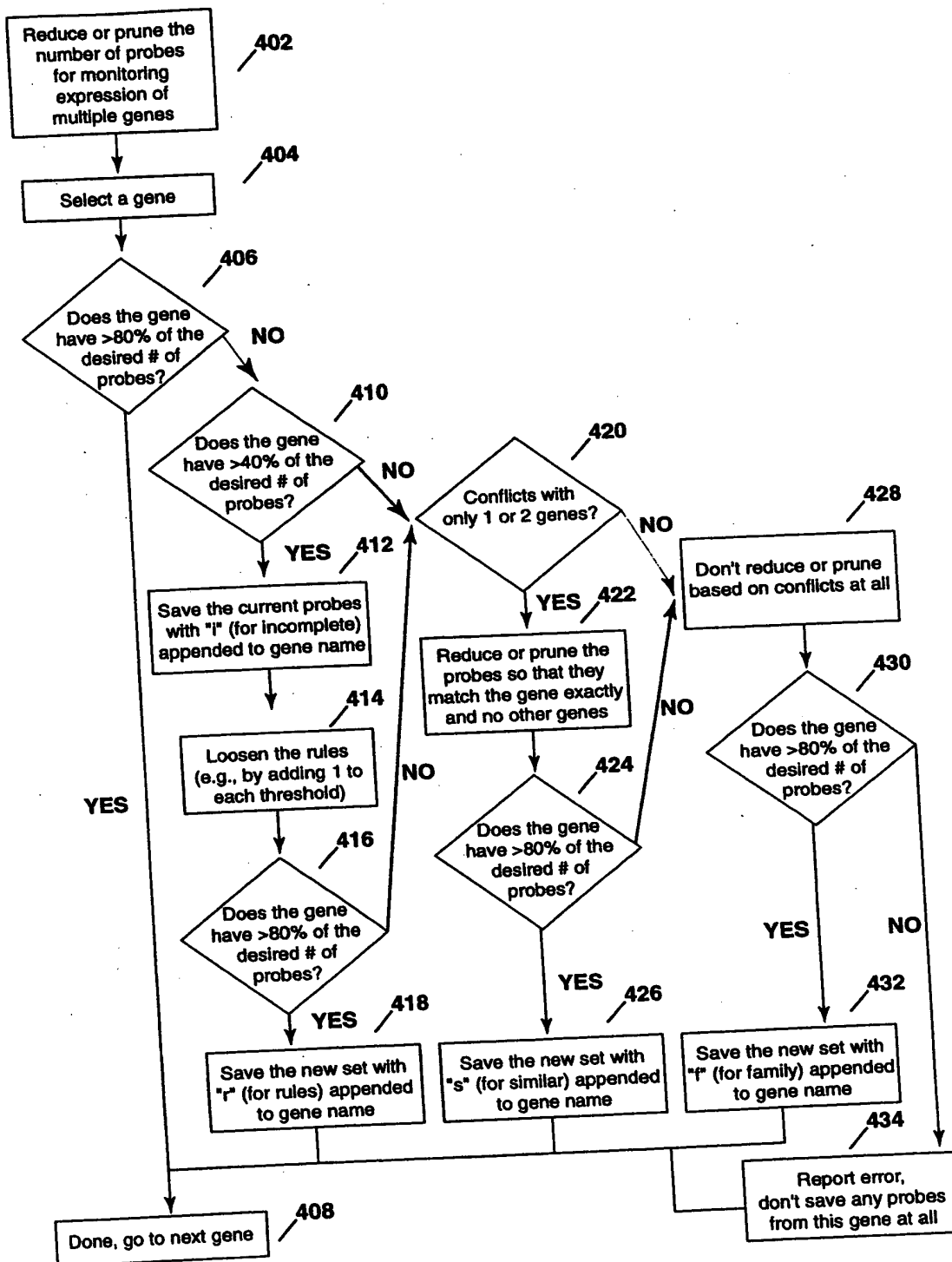


Figure 11

# Discrimination with Ligation

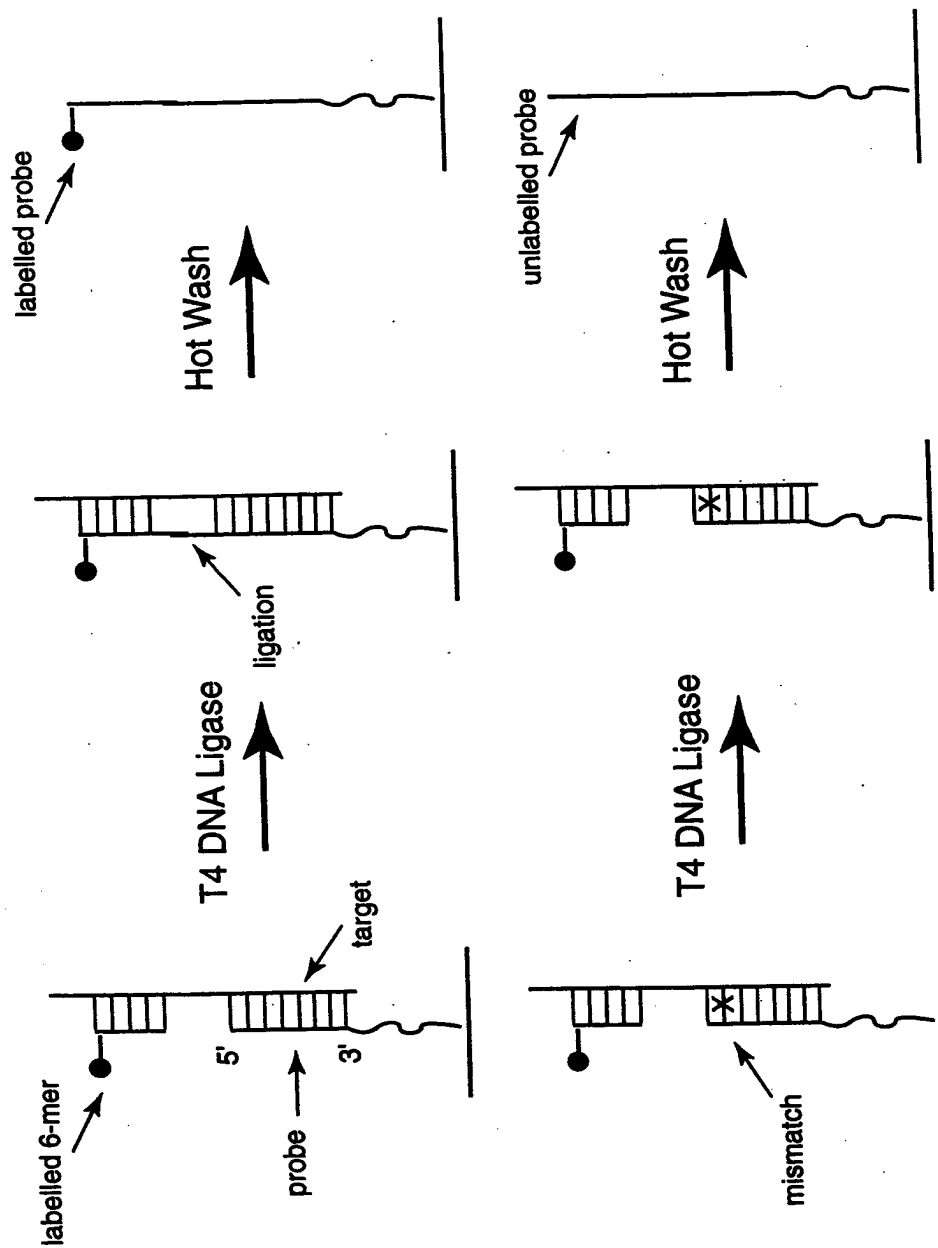


Figure 12

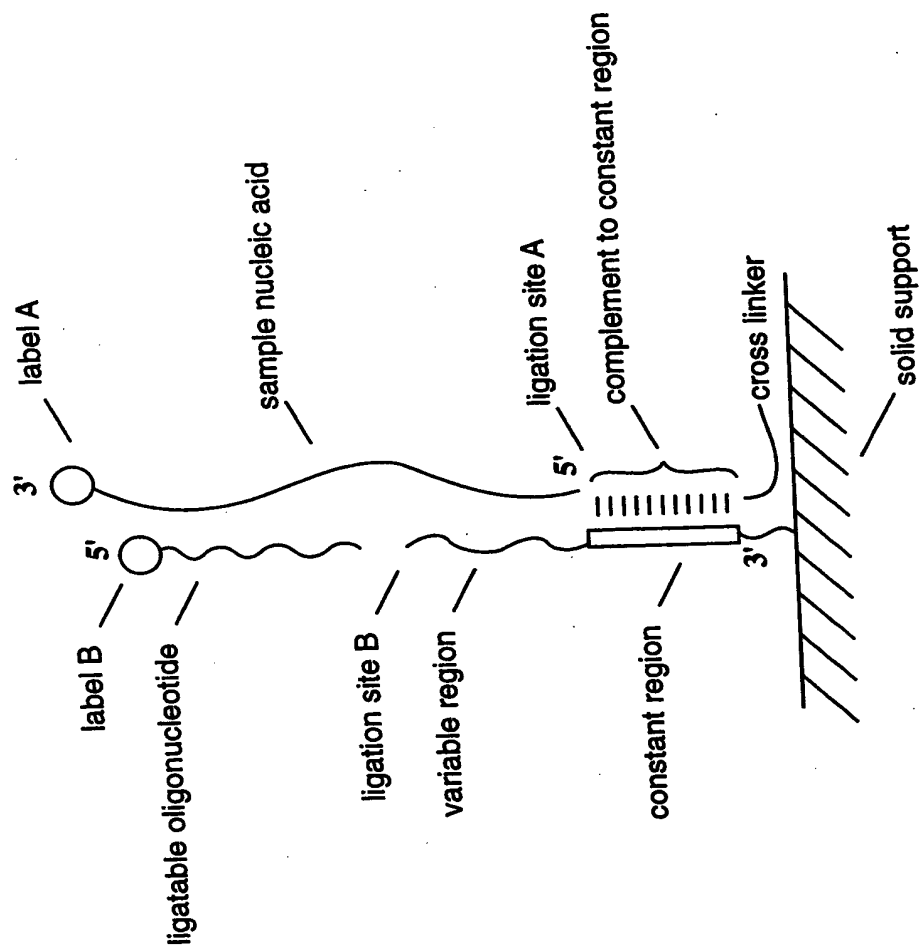


Figure 13a







Figure 14a

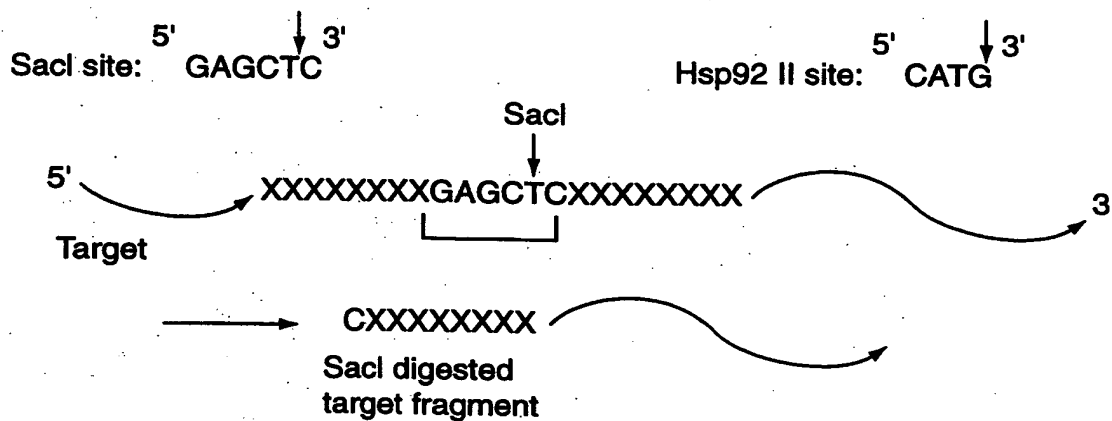
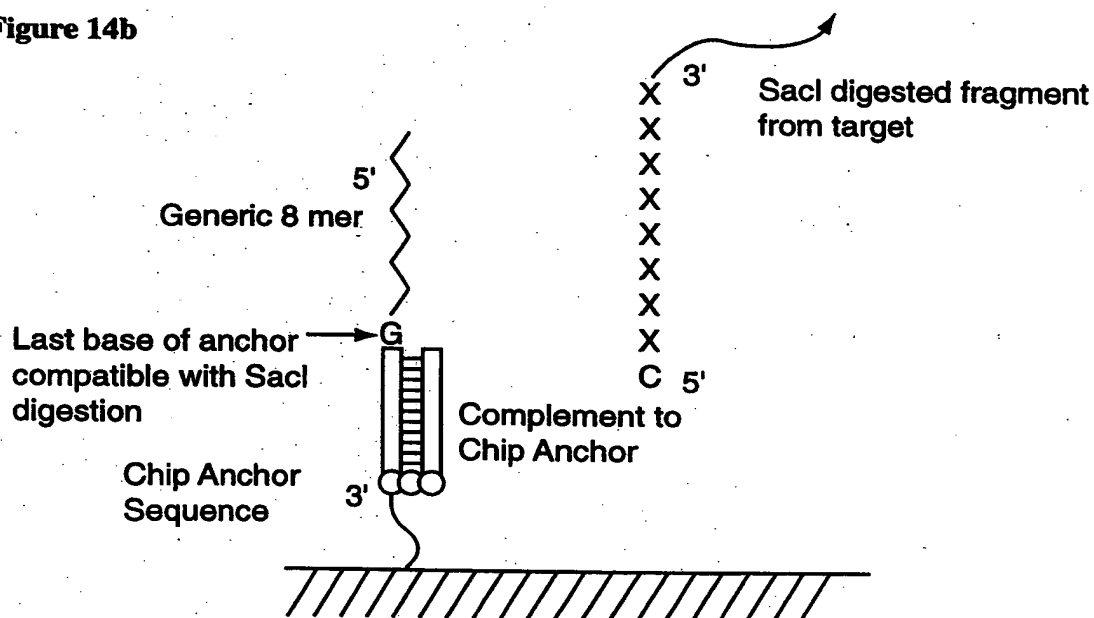


Figure 14b



**Figure 14c**

Monitoring mRNA expression from organisms with small genomes:

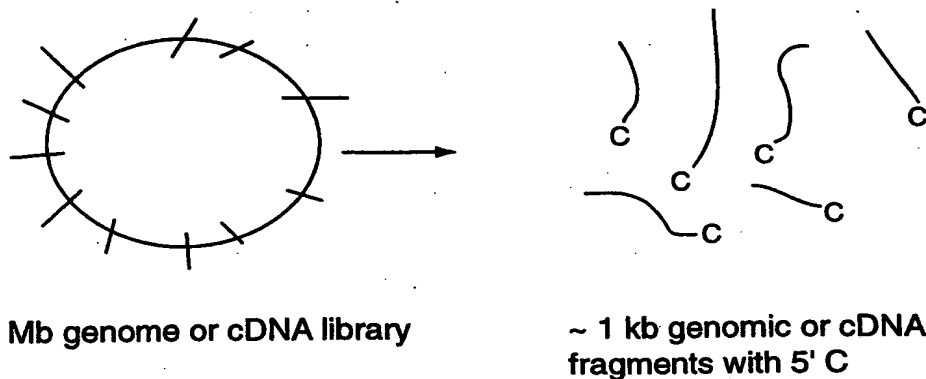
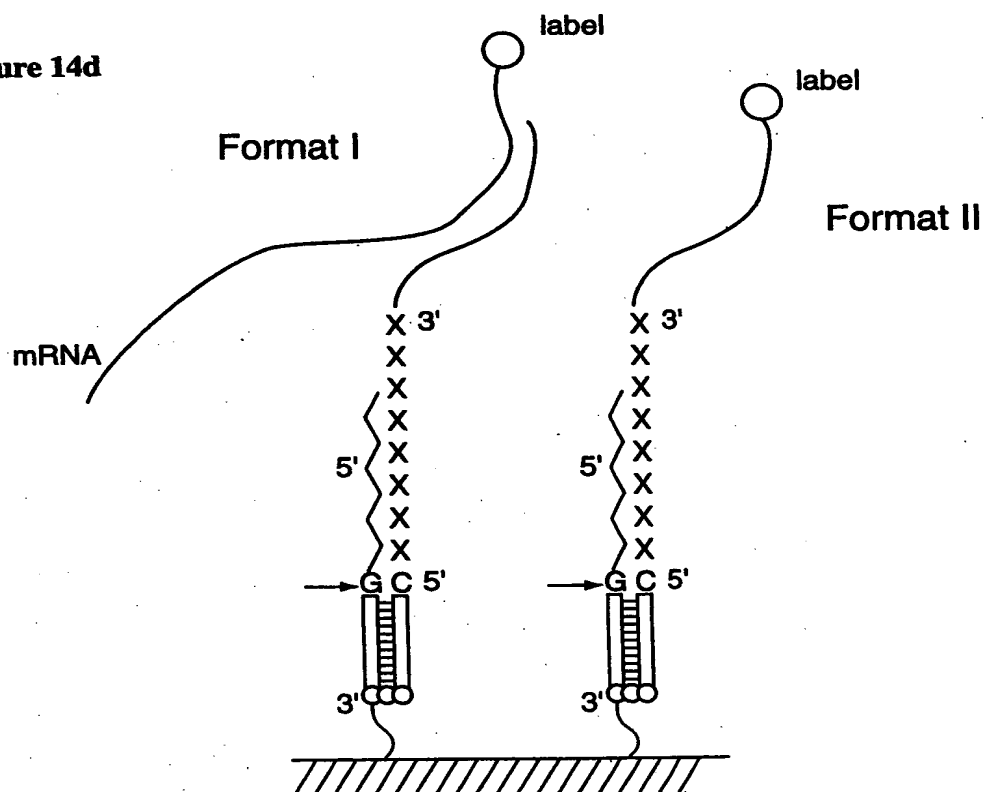
**Figure 14d**

Figure 15a

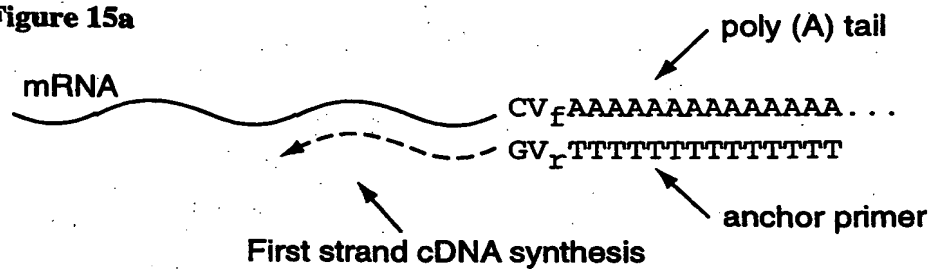


Figure 15b

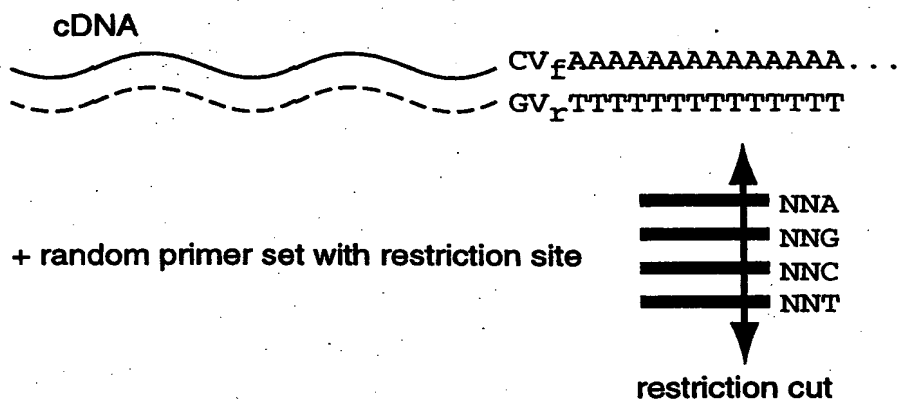


Figure 15c

random primer PCR of cDNA

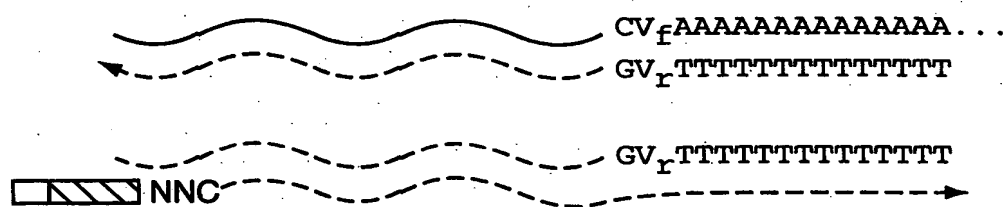


Figure 15d

Restriction digest PCR products



Figure 15e

Sort fragments by 5' ends on Generic Ligation GeneChip

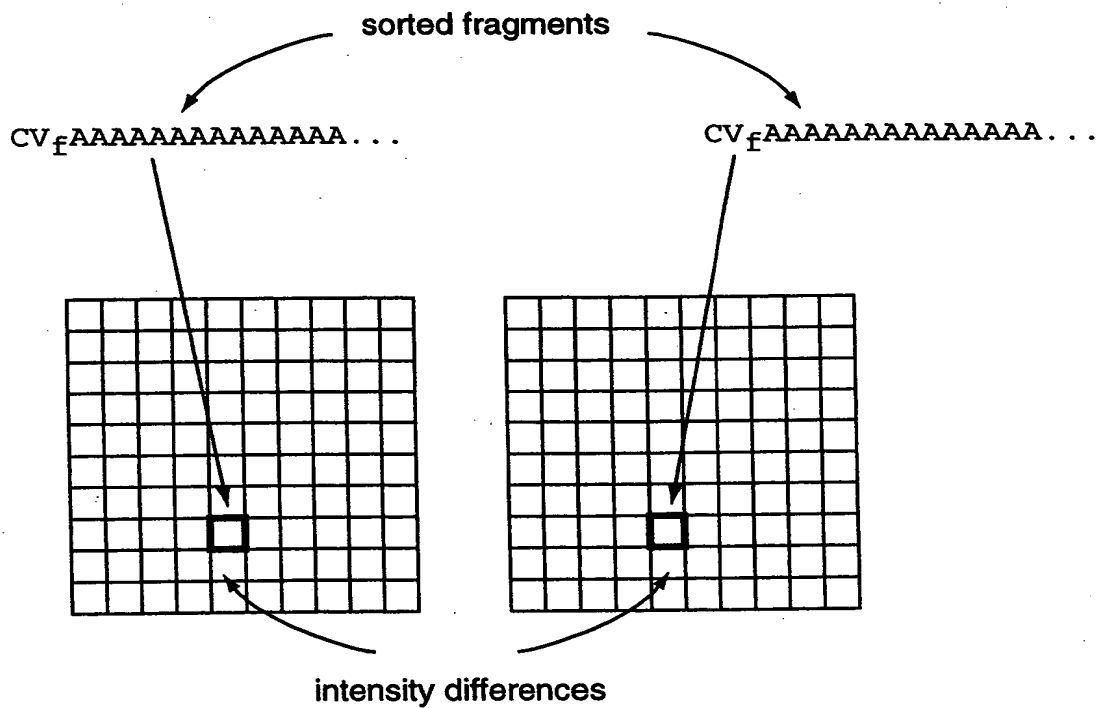
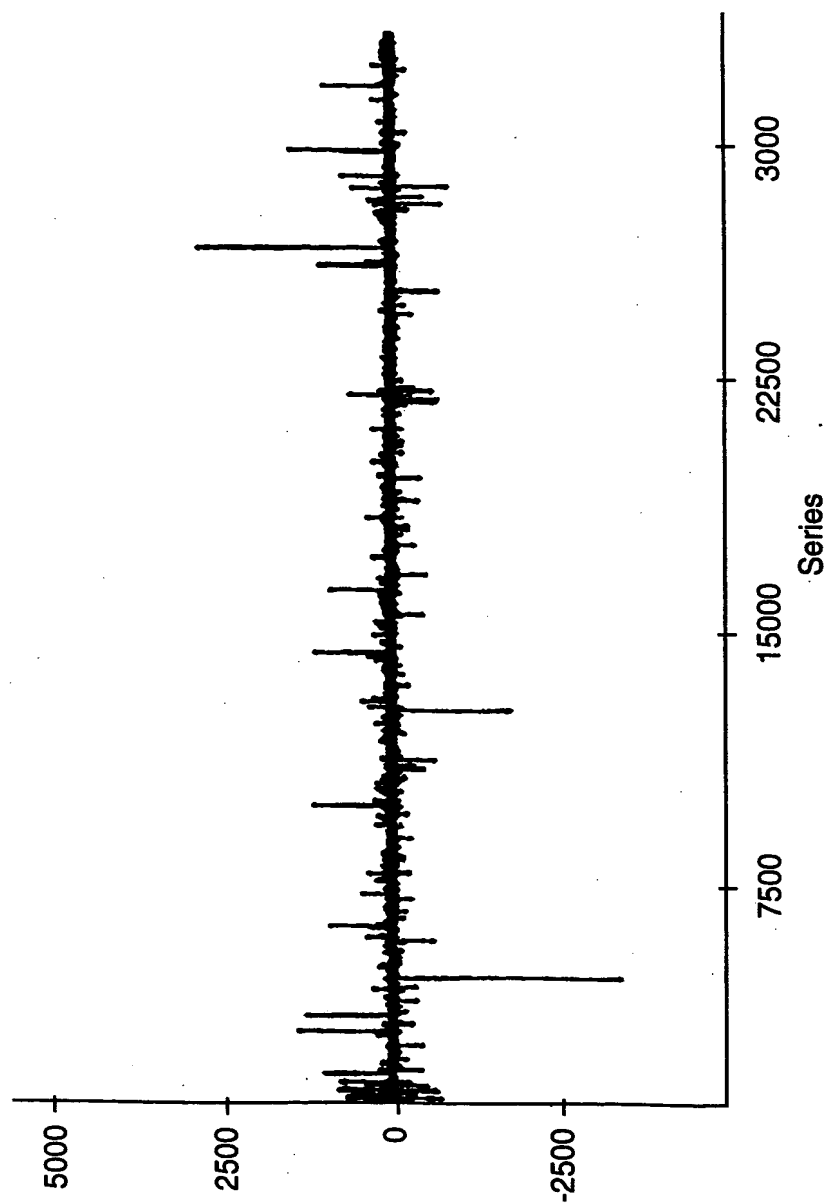


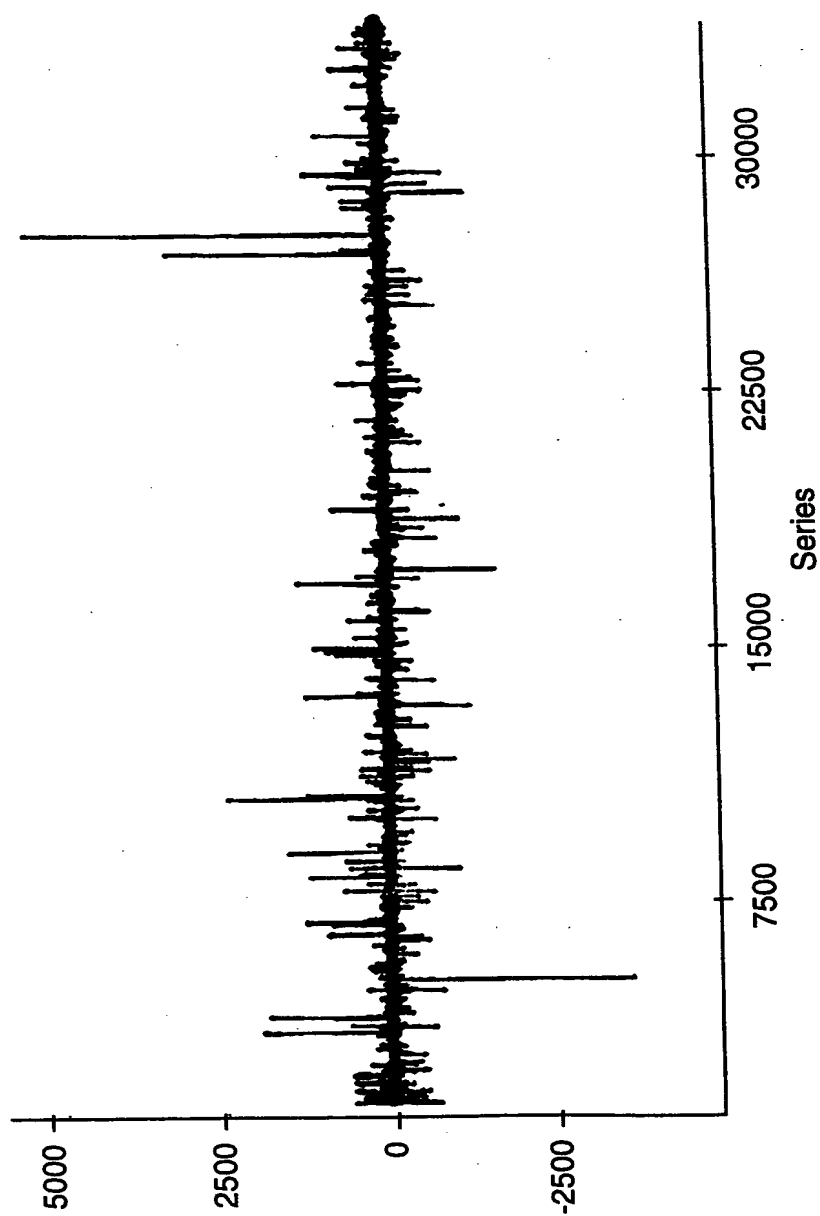
TABLE 22-20000



Sample 1 vs. Sample 1 - Absolute Differences  
(Replicate 1 vs. Replicate 2)

Figure 16a

703739 4223333



Sample 2 vs. Sample 2 - Absolute Differences  
(Replicate 1 vs. Replicate 2)

Figure 16b

100190 22200000

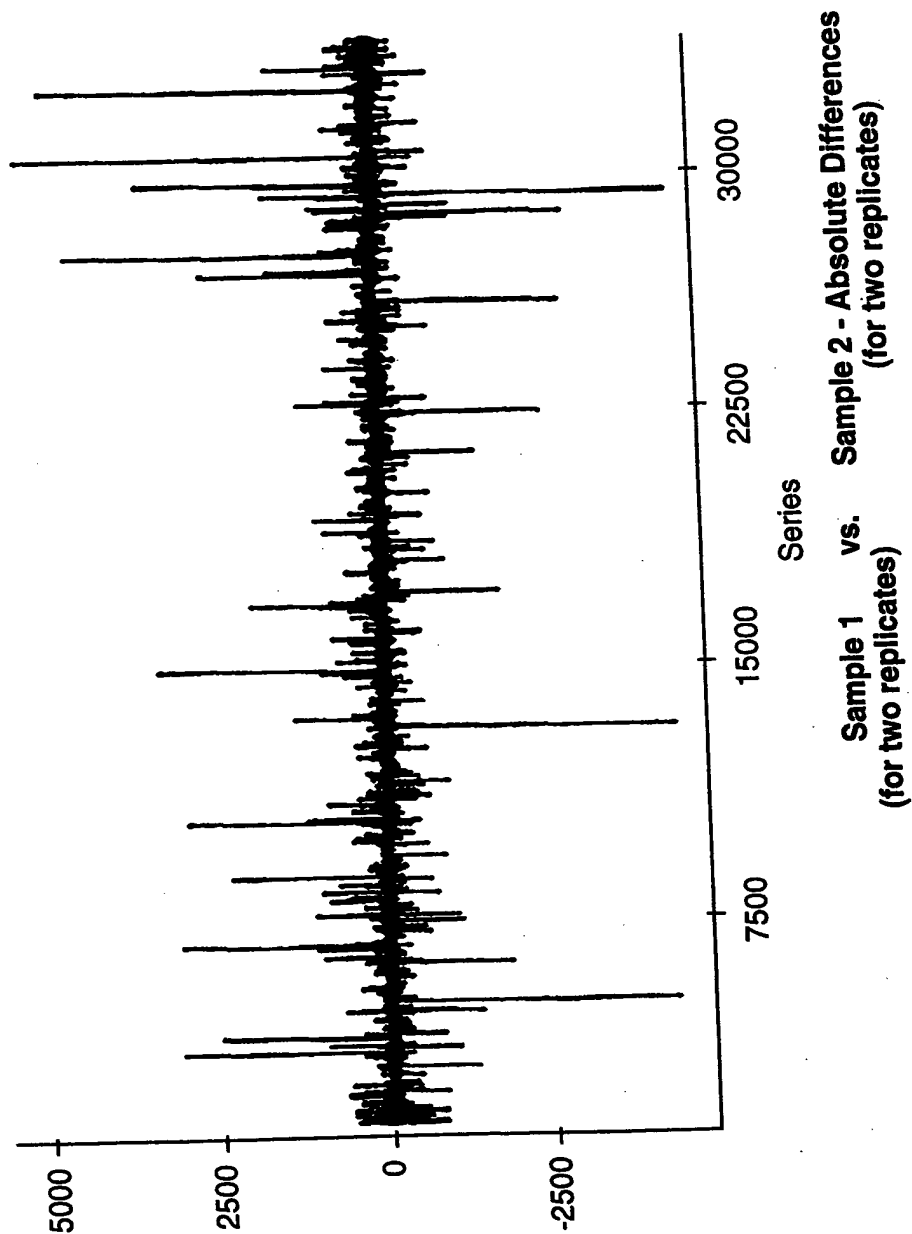
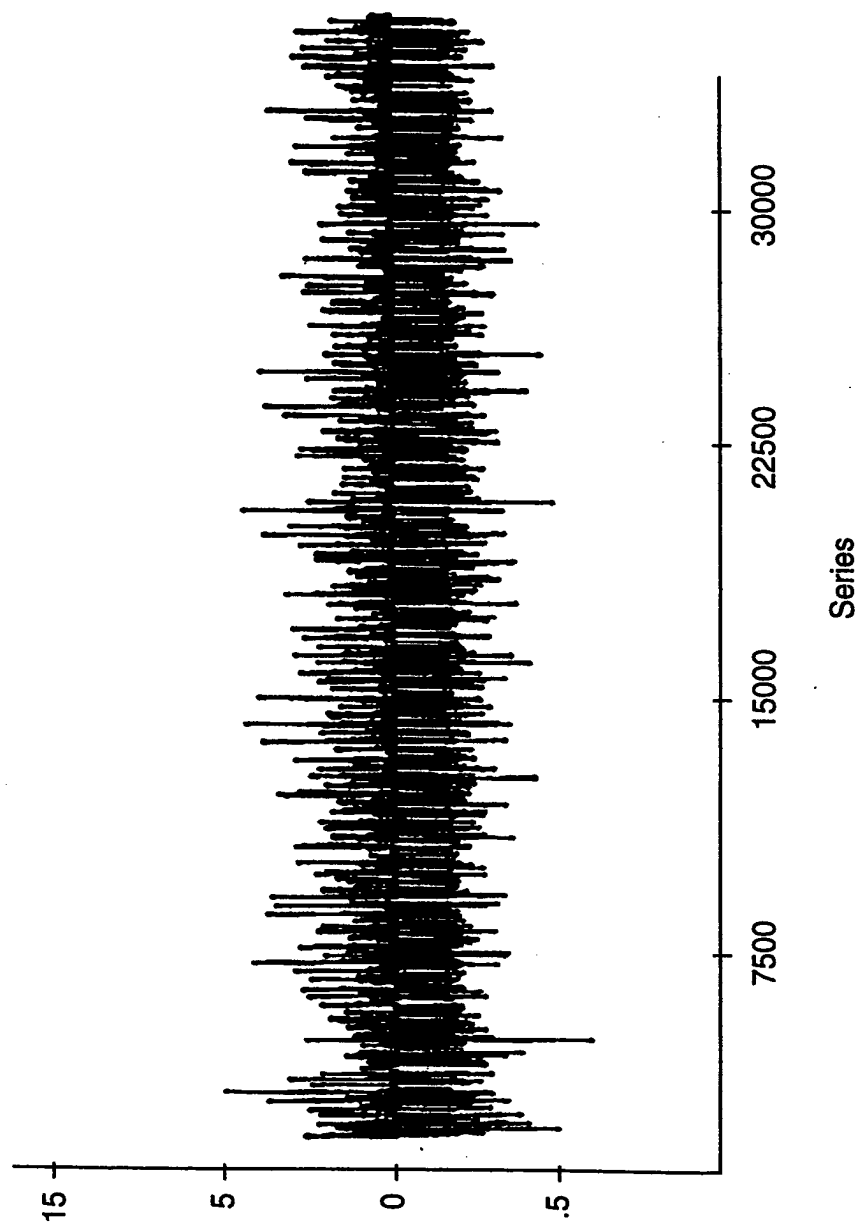


Figure 16c





REP 1 22400000



Sample 2 vs. Sample 2 - Ratios  
(Rep 1) (Rep 2)

Figure 17b

702130 220000

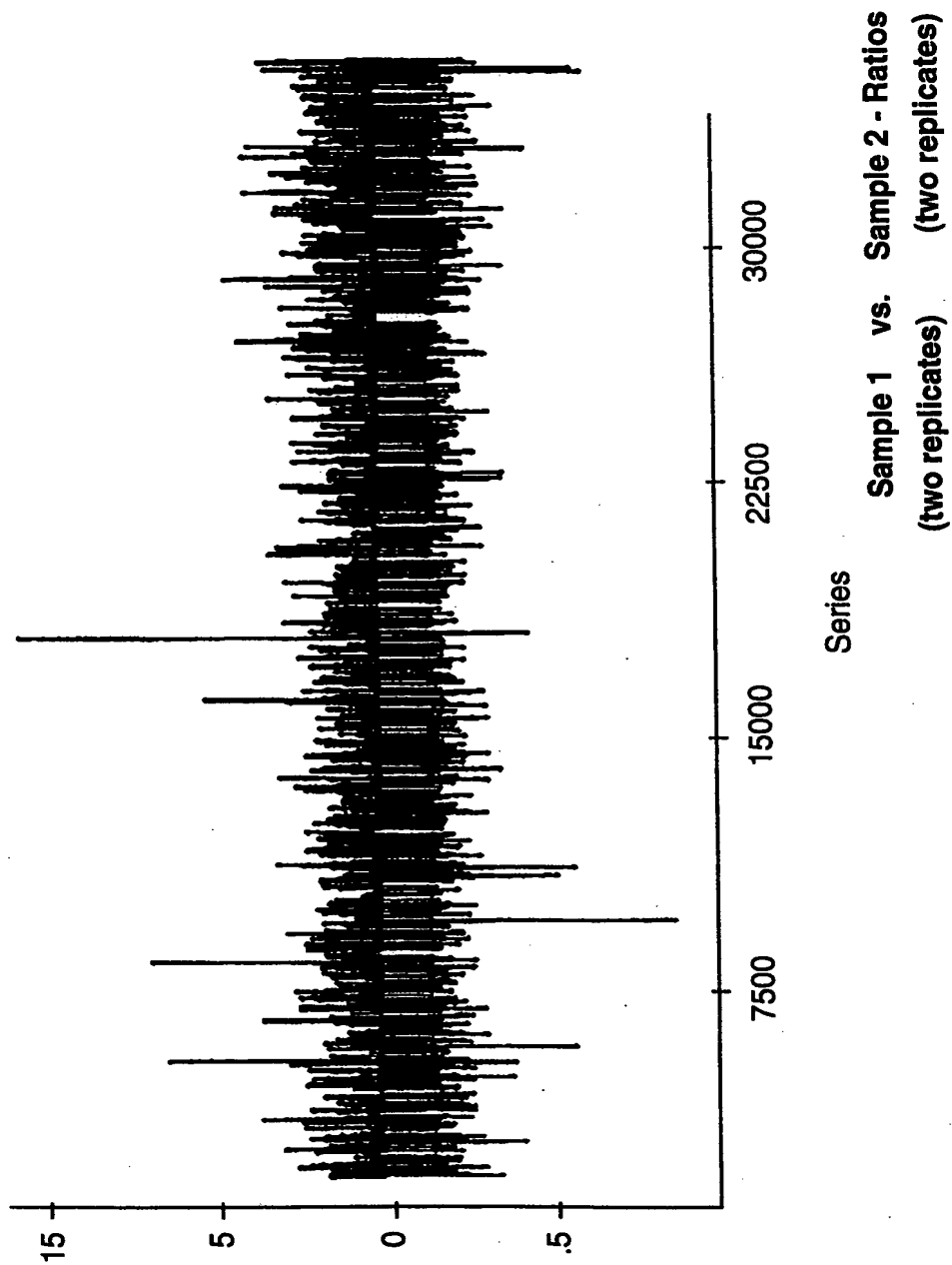
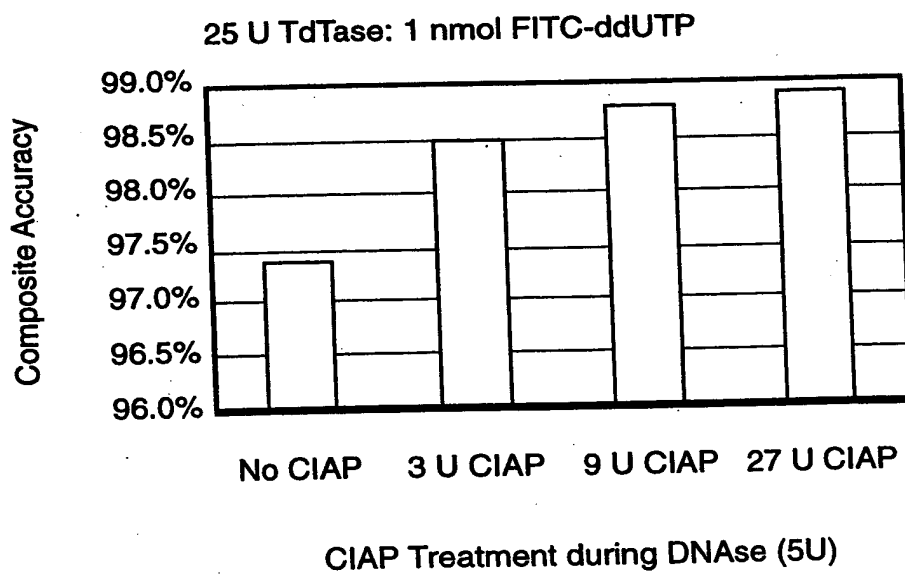
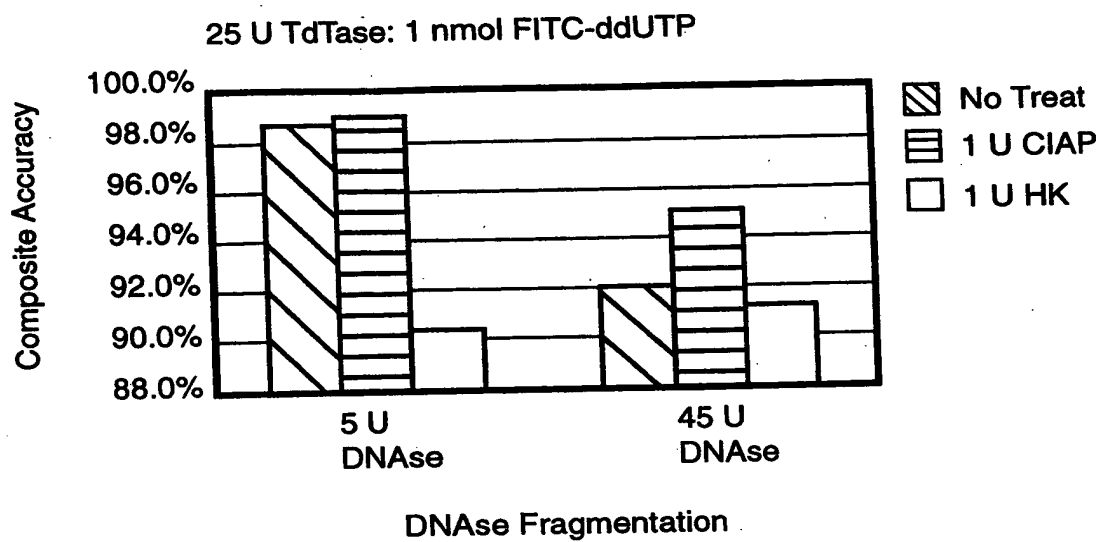


Figure 17c

**Post-Fragmentation End Labeling: CIAP Treatment****Figure 18**

## Post-Hybridization End Labeling on the Chip

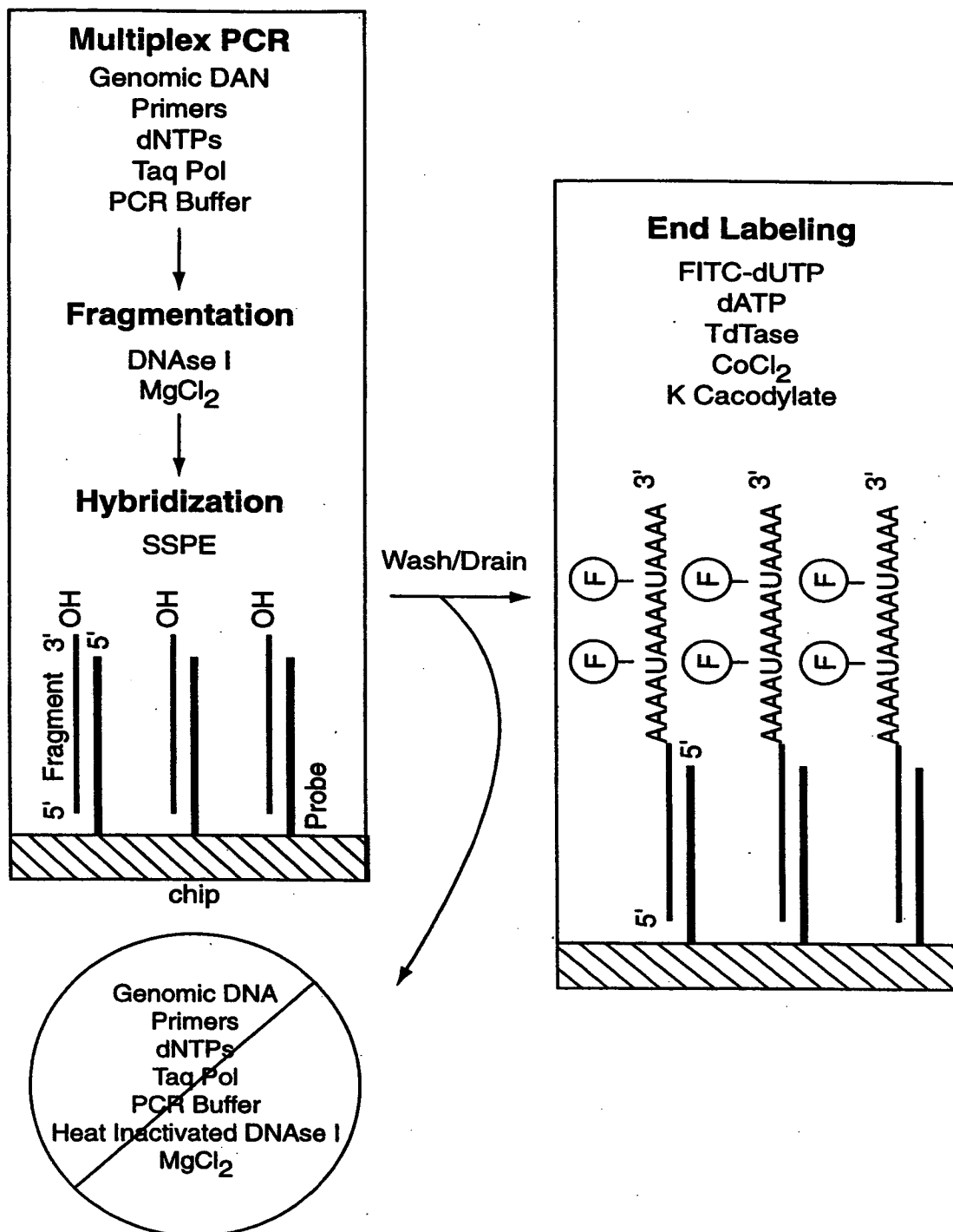


Figure 19

# Pre-react Chip Prior to Hybridization and Labeling

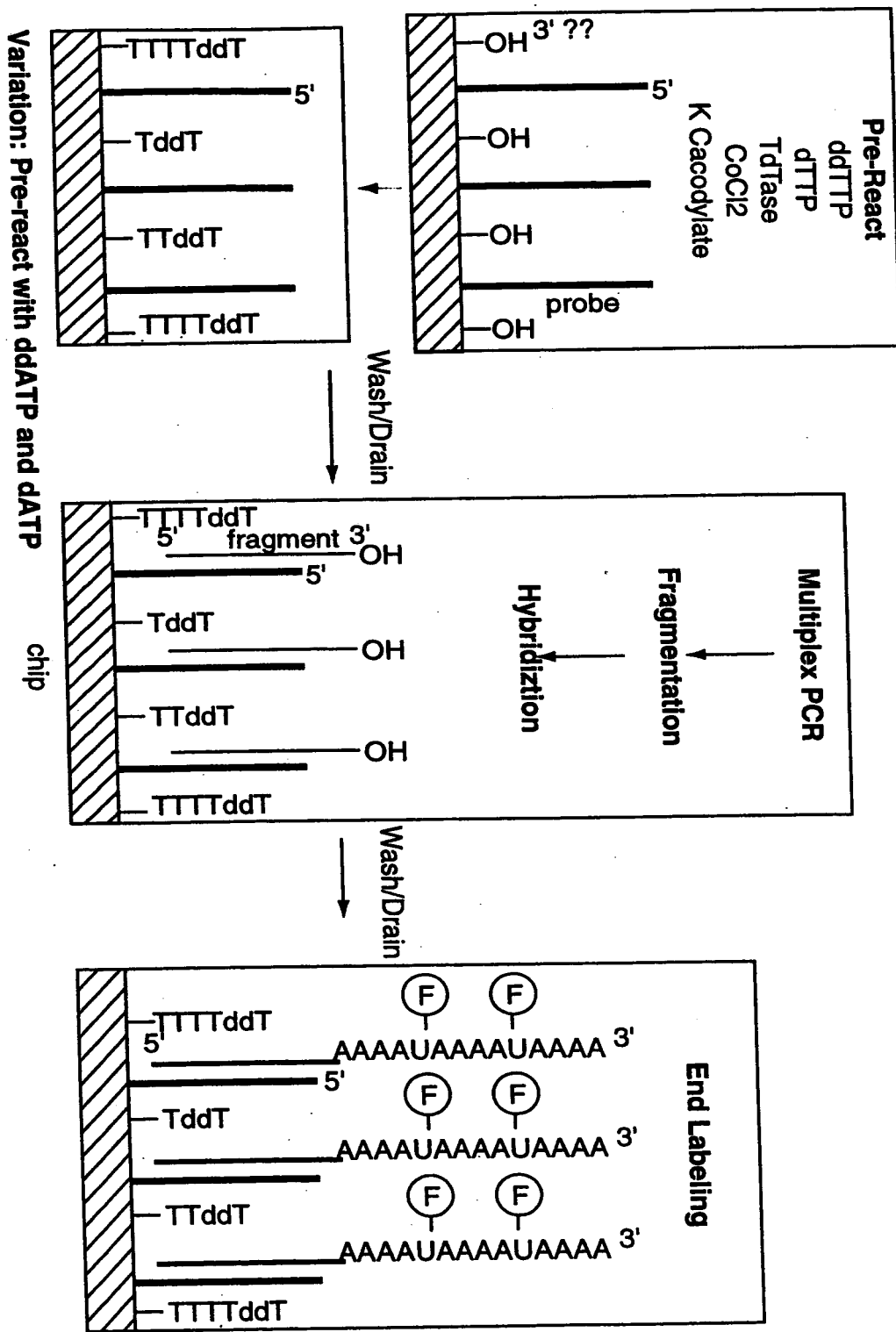


Figure 20

00000727 061301

## DNase Titration: "Ideal" Fragment Length

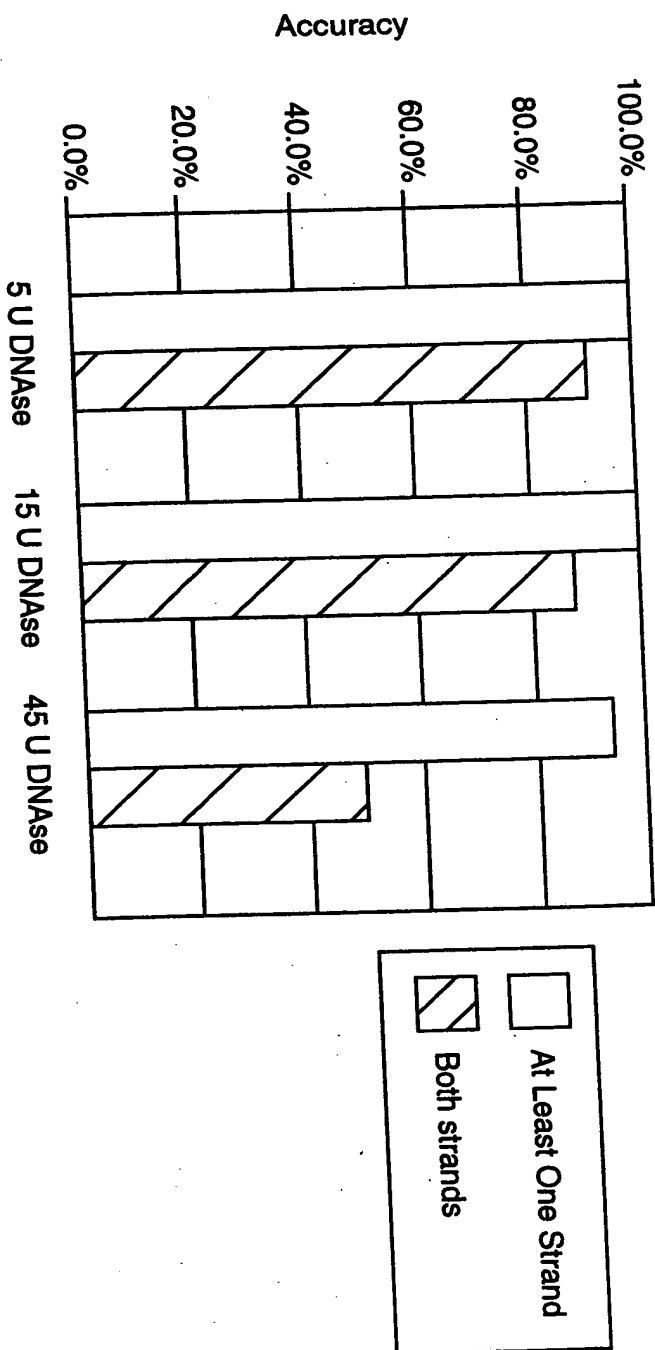
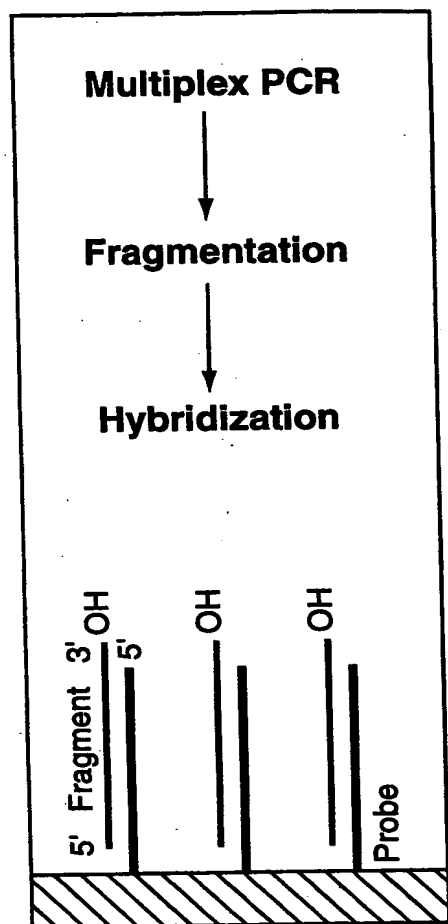


Figure 21

00000000 00000000

## Oligo dT Labeling on the Chip



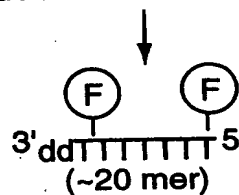
chip

Substitute FITC with:

- Rhodamine R110
- Cy fluorochrome

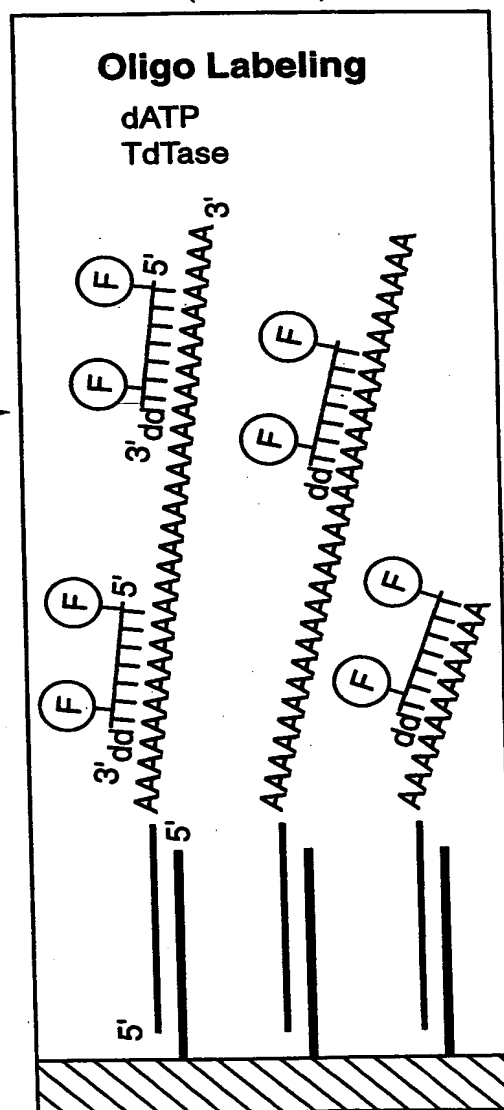
## Oligo Synthesis

FITC-Phosphoramidite  
ddT nucleoside at 3' end  
dT nucleosides



## Oligo Labeling

dATP  
TdTase



Wash/Drain

Figure 22



## Labeling Reagents

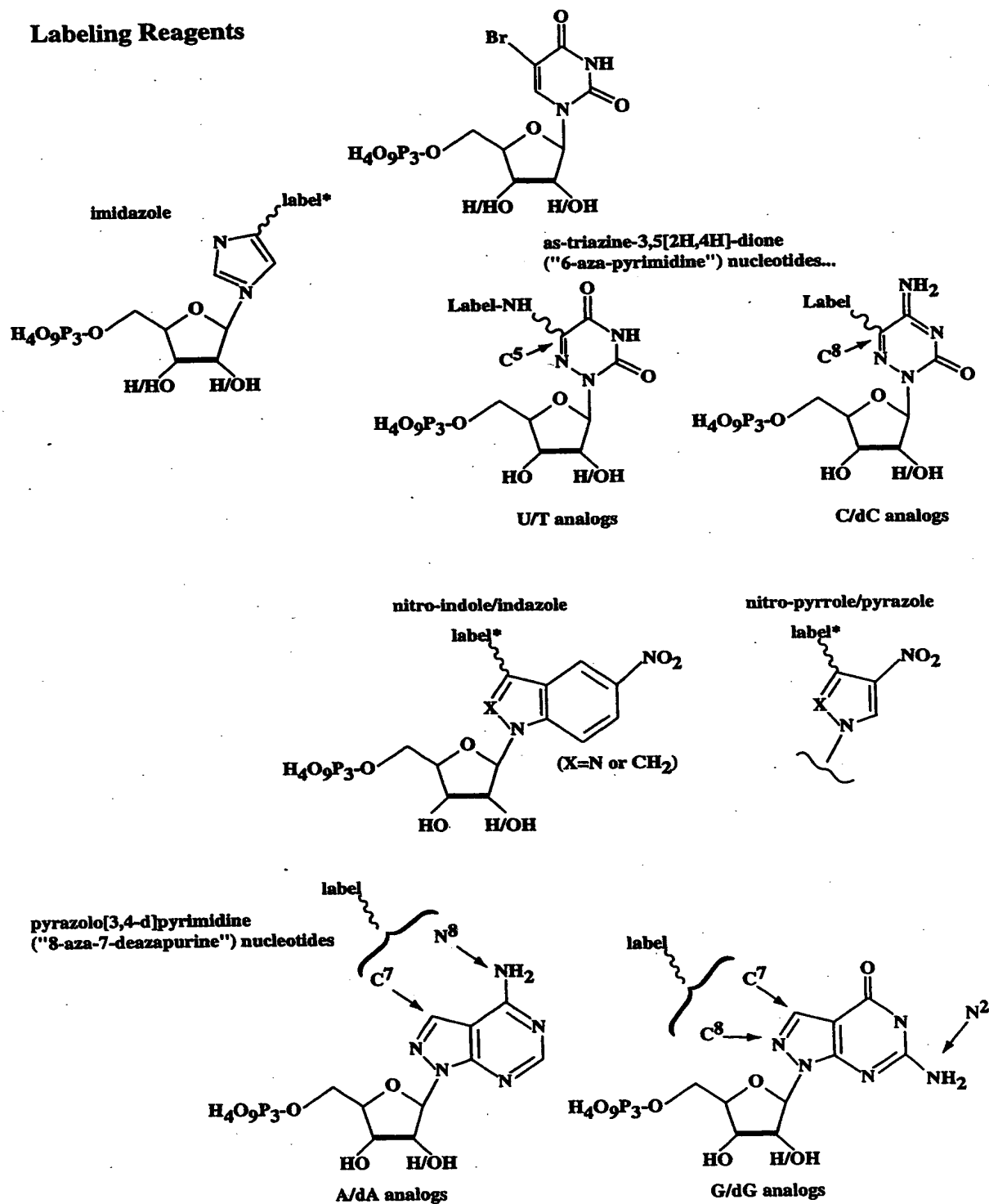


Figure 23a

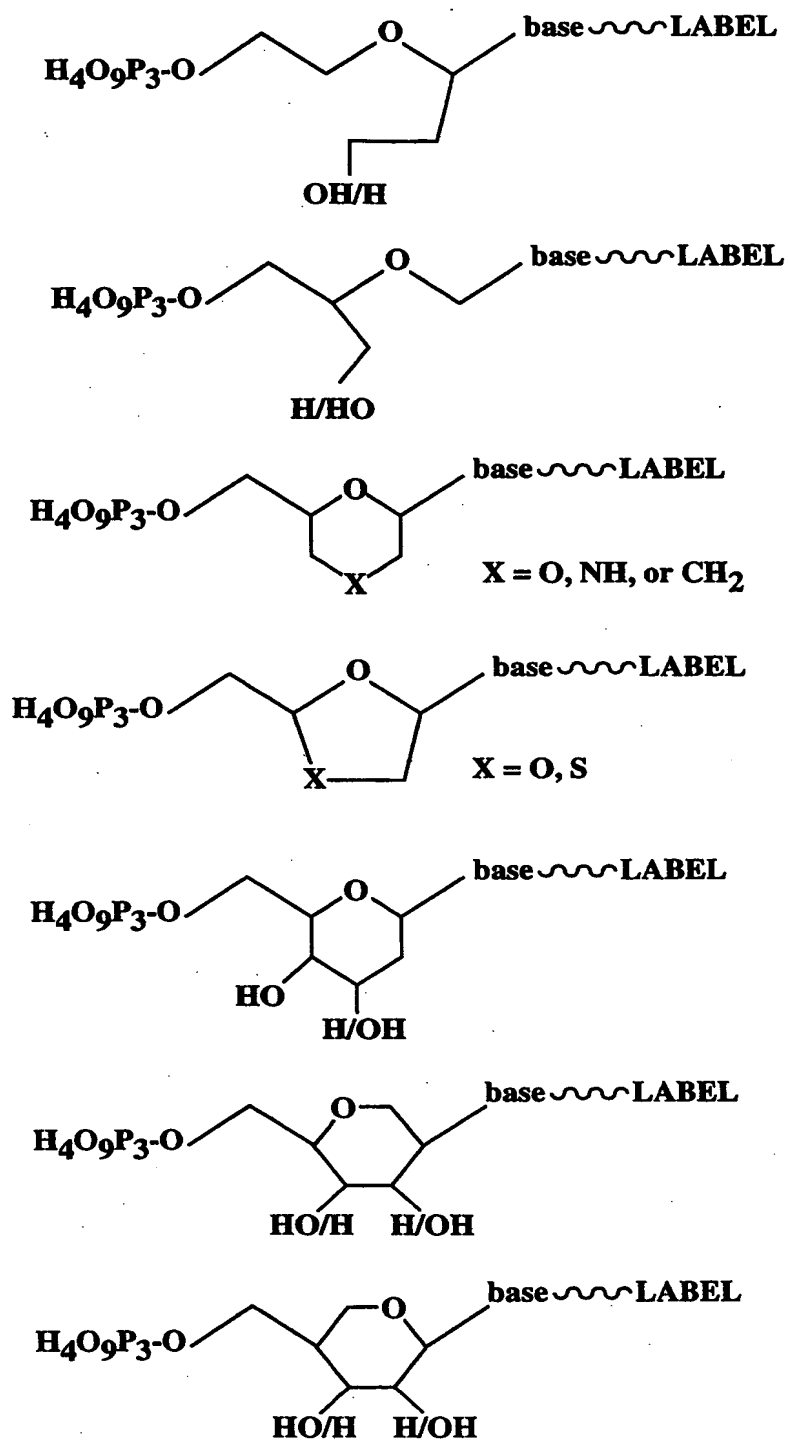
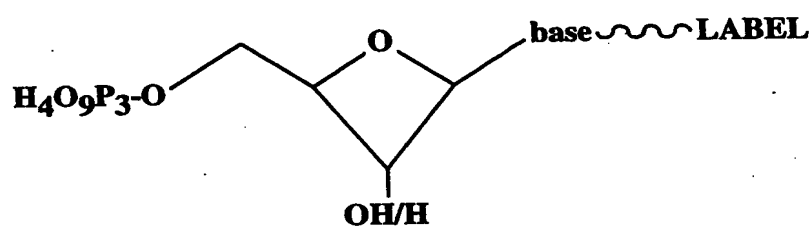
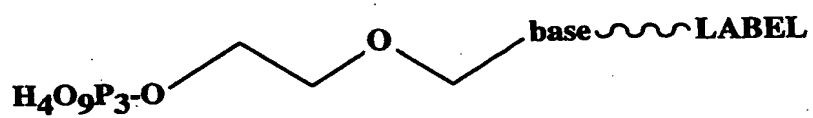


Figure 23b



base = heterocyclic moiety (eg. analogs thereof)

~~~~~ = linker;

LABEL = detectable signal-gene

Figure 23c

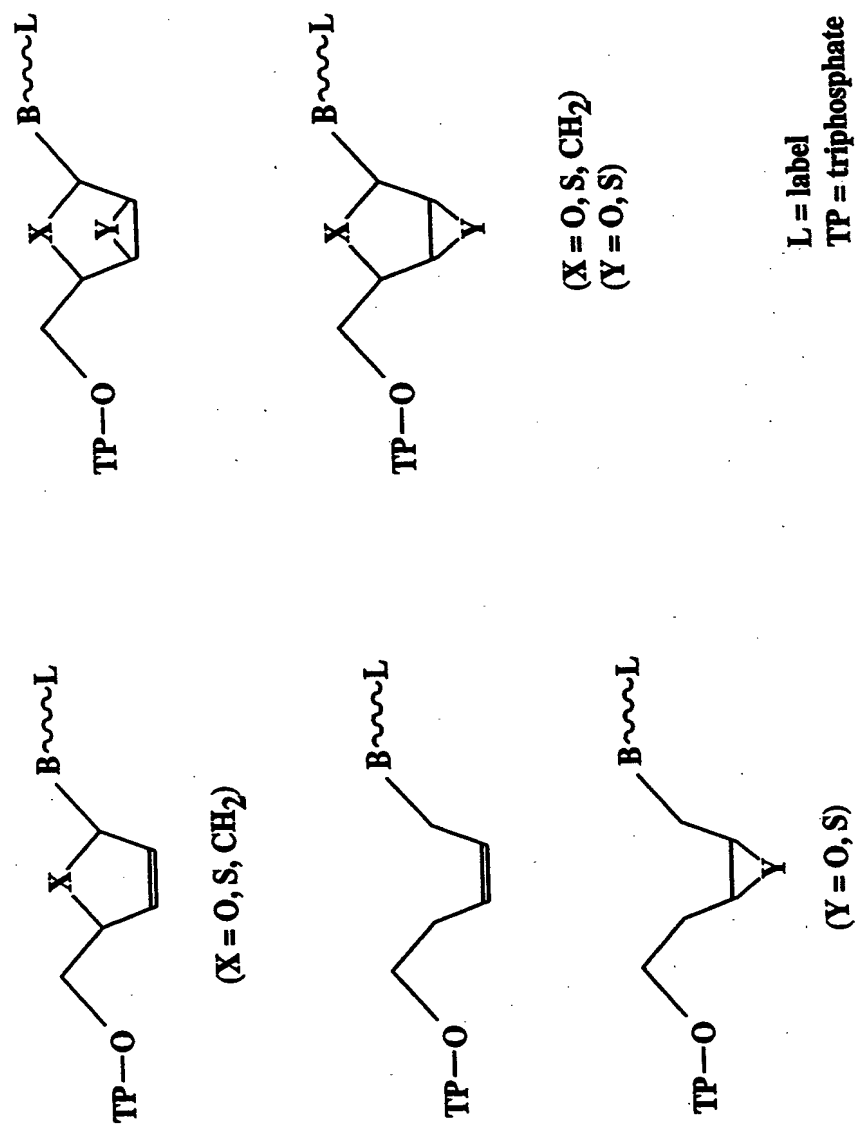


Figure 23d

## Resequencing a target DNA molecule with a set of generic n-mer tiling probes

**ie. 4-mer probes:**

**Target:** 5' TGACATAGCACGAGGGA... 3'

Probe 1: ACTG<sup>5'</sup>

Probe 2: CTGT

**Probe 3: TGTA**

**GTAT**

**Probe 5:** TATC

**ATCC**

**TCC**

CCTG

Probe 9:  
CTGT...etc.

**37147**

**Figure 24**

Four electronic tiling arrays are present on a 4-mer generic array:

(4 x 3 = 12 "nearest neighbors" for each probe)

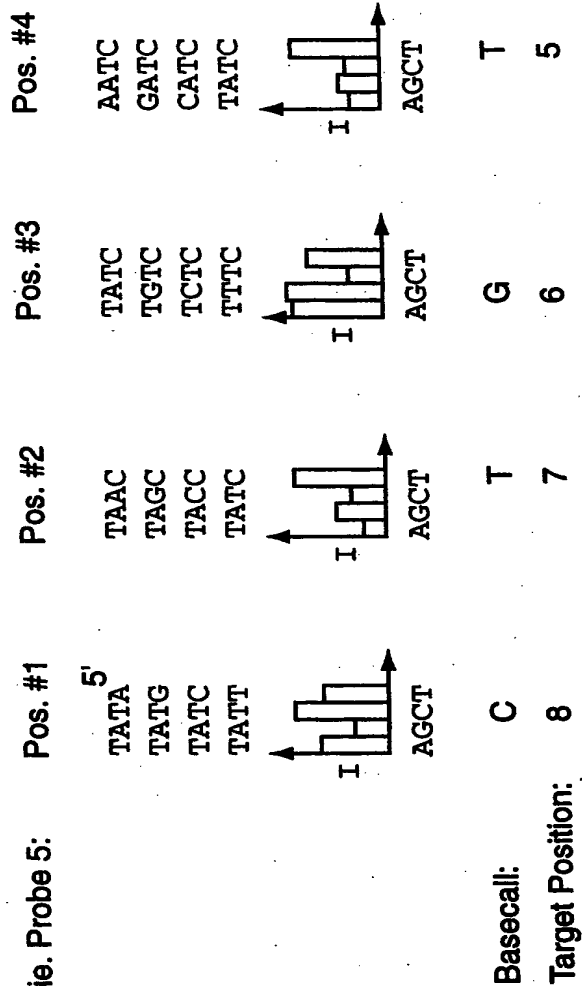


Figure 25

Base Calling at the 8<sup>th</sup> position in the target

|                 | 8 <sup>th</sup>          |                 |
|-----------------|--------------------------|-----------------|
| Target:         | TGACATAGGACAGCGAAGGGA... | Base-Call       |
| Probe 5, Pos. 1 | 3' TATC 5'               | T               |
| Probe 6, Pos. 2 | ATCC                     | G               |
| Probe 7, Pos. 3 | TCCT                     | C               |
| Probe 8, Pos. 4 | CCTG                     | C               |
|                 |                          | <hr/>           |
|                 |                          | C is the winner |

Figure 26

Base Vote Table

| Base<br>Position | Base<br>Identity | Base<br>Vote | Correctness<br>Score Total | Correctness<br>Score #1 | Correctness<br>Score #2 | Correctness<br>Score #3 | Correctness<br>Score #4 |
|------------------|------------------|--------------|----------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| 5                | T                | T            | 1                          | 1                       | 0                       | 1                       | 0                       |
| 6                | A                | A            | 1                          | 1                       | 1                       | 1                       | 1                       |
| 7                | T                | T            | 1                          | 0                       | 1                       | 0                       | 1                       |
|                  |                  |              |                            |                         |                         |                         |                         |
| 9                | C                | G            | 0                          | 0                       | 0                       | 0                       | 0                       |
| 10               | T                | T            | 1                          | 1                       | 0                       | 1                       | 0                       |
| 11               | G                | G            | 1                          | 0                       | 1                       | 1                       | 1                       |
| 12               | T                | T            | 1                          | 0                       | 1                       | 1                       | 1                       |
| 13               | C                | C            | 1                          | 1                       | 0                       | 0                       | 1                       |
| 14               | G                | G            | 1                          | 0                       | 1                       | 1                       | 1                       |
| (10) TOTALS      |                  |              |                            |                         |                         |                         |                         |

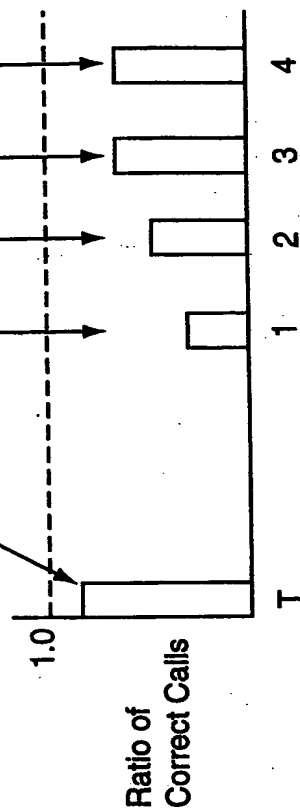


Figure 27



# Effect of Applying Correctness Score Transform to HIV Data

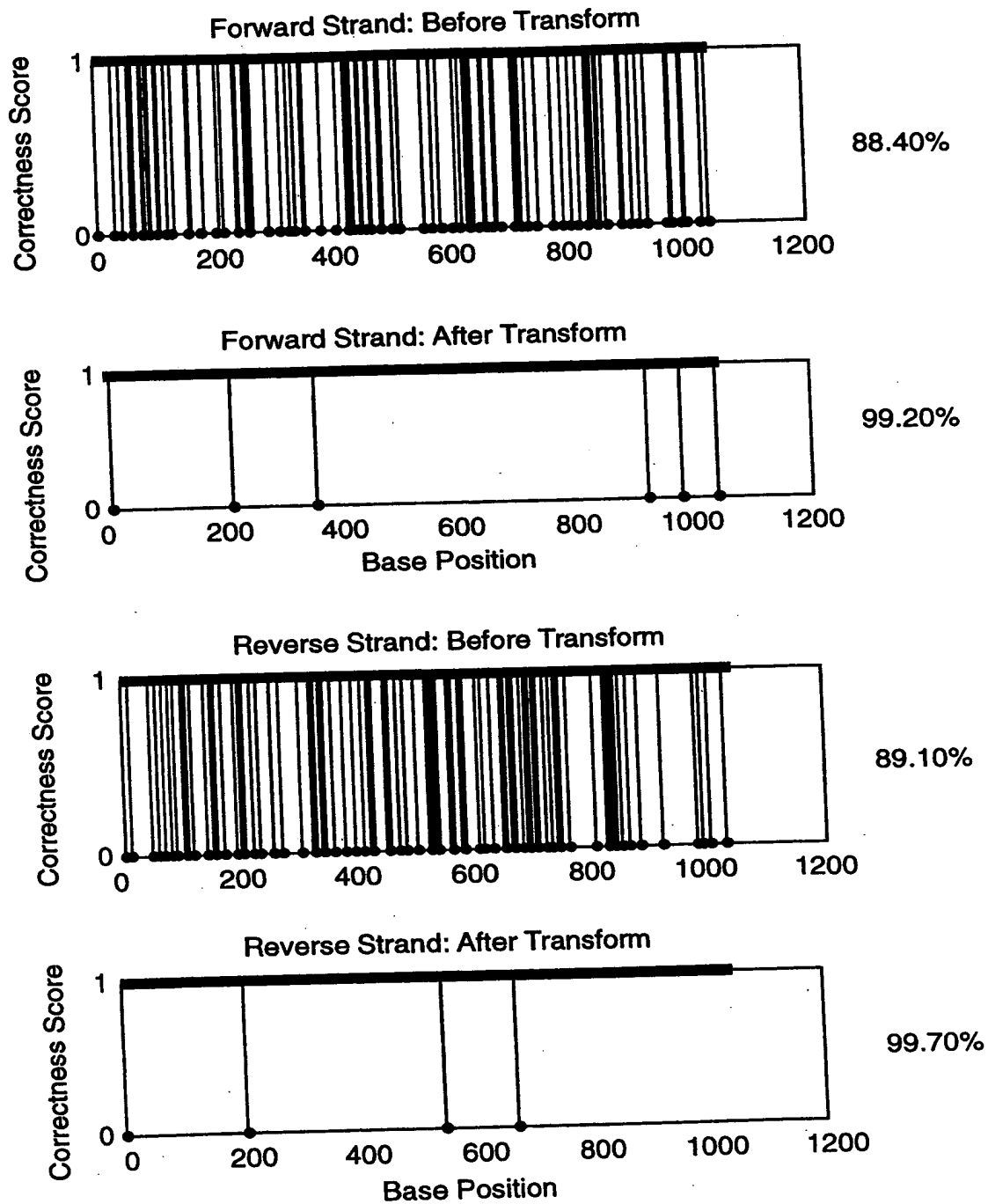
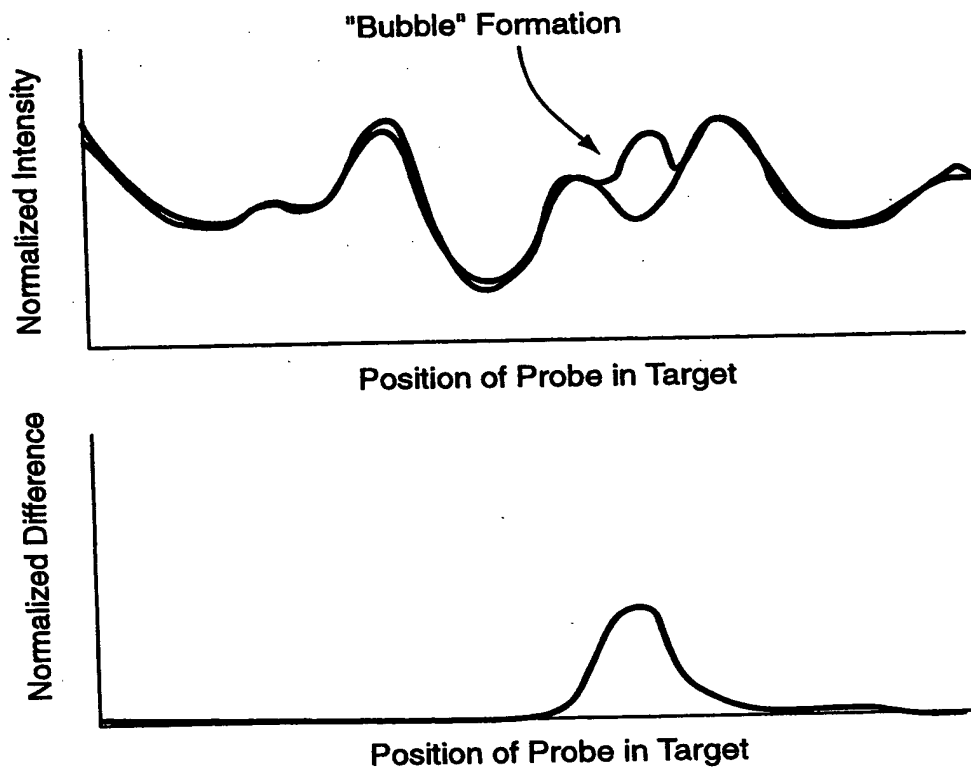


Figure 28

# Mutation Detection by Intensity Comparisons



## Algorithms:

$$I_{\text{normalized}} = I_{\text{probe}} / (\sum I_{\text{NN}})$$

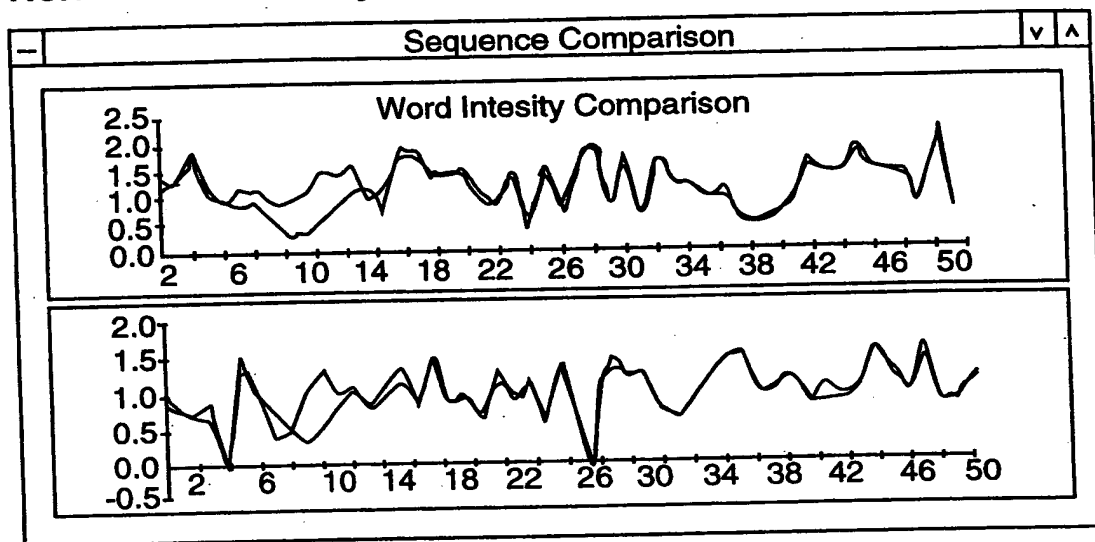
$$I_{\text{difference}} = \frac{(I_{\text{normalized, variant}} - I_{\text{normalized, control}})}{(I_{\text{normalized, variant}} + I_{\text{normalized, control}})}$$

- Locally normalized intensities track well
- Local normalization is sensitive to mutations

Figure 29

## Bubble Formation Detection of Mutation in HIV Genome

## Normalized Intensity Comparison:



## Normalized Difference:

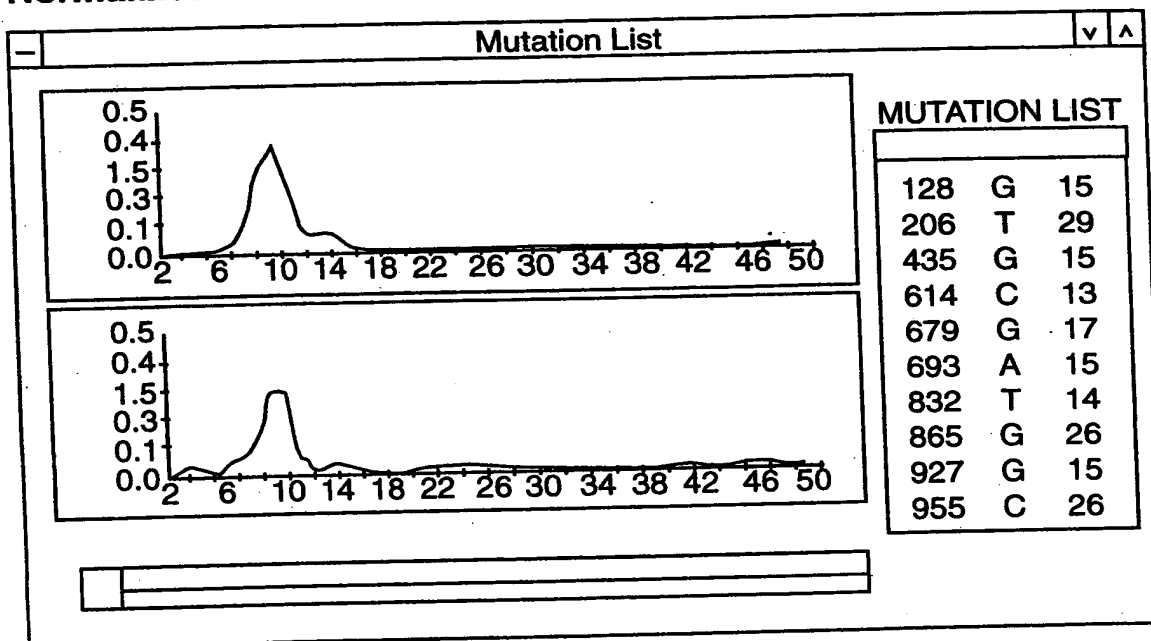
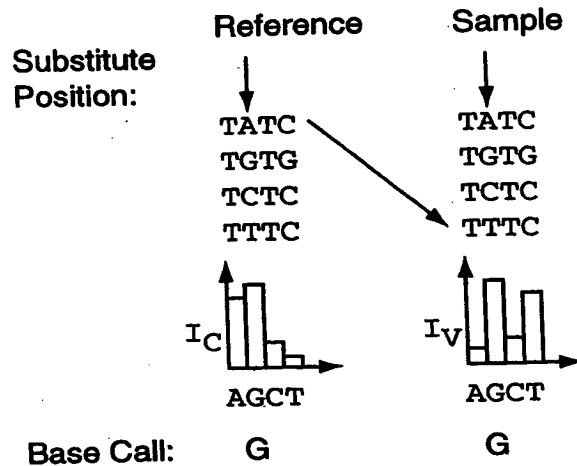


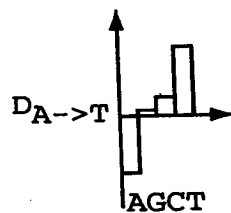
Figure 30

## Induced Difference Nearest Neighbor Probe Scoring:



$$\text{Induced Difference: } D_A = (I_{V,A} - I_{C,A}) / I_{C,A}$$

- Average induced differences over all tilings and over both forward and reverse strands.



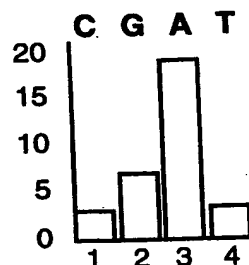
- Probe with A - "down-regulated"
- Probe with T - "up-regulated"
- A  $\rightarrow$  T mutation

- Total Induced Difference > + Threshold: Mutation Exists
- Total Induced Difference < - Threshold: Mutation Exists
- Two criteria for mutations: Induced Difference Scores; Bubble Formation

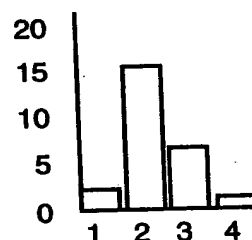
Figure 31

**Mutations found in an HIV PCR target (B) using a generic ligation  
GeneChip and induced difference analysis**

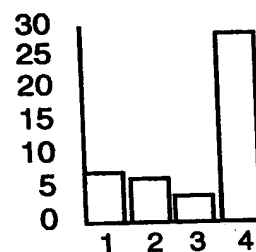
21                      30                      40                      50  
actgtatccttttagcttcctcagatcact  
actgtatcctttaacttcctcagatcact



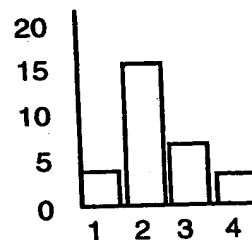
134              140                      150                      160  
attagaagaaatgaatttgccaggaagatg  
attagaagaaatgagtttgccaggaagatg



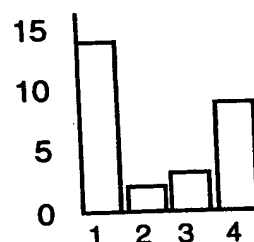
211                      220                      230                      240  
agtatgatcagatacccatagaaatctgtg  
agtatgatcagatactcatagaaatctgtg



440                      420                      430                      440  
agaaatttgtagacagaaatggaaaaggaagg  
agaaatttgtagacagrgatggaaaaggaagg

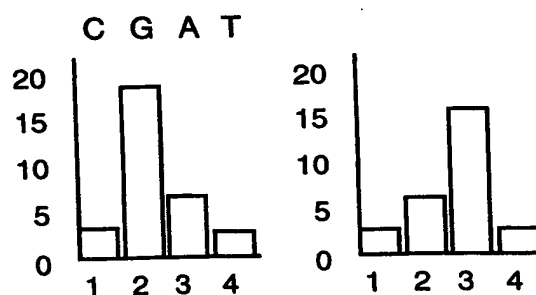


621                      630                      640                      650  
catcccgagggttaaaaaagaaaaaatca  
catcccgagggtcmaaaaaagaaaaaatca

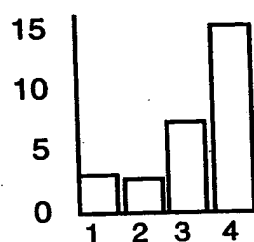


**Figure 32a**

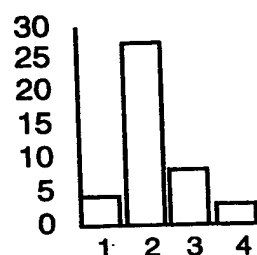
693      700              710              720  
 ttagataaagacttcaggaagtataactgca  
 ttagatgaagacttcaggaaatataactgca



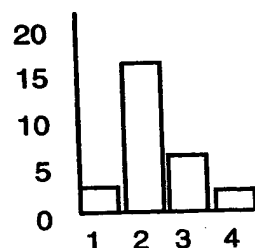
840              850              860              867  
 tagagccttttagaaaaacaaaatccagaca  
 tagagccttttagataacaaaatccagaca



872              880              890              900  
 tatctatcaatacatggatgatttgatgt  
 tatctatcaatacgtggatgatttgatgt



934      940              950              960  
 caaaaatagaggaactgagacaacatctgt  
 caaaaatagaggagctgagacaacatctgt



960              970              980              989  
 ctgttgaggtggggatttaccacaccagac  
 ctgttgaggtggggacttaccacaccagac

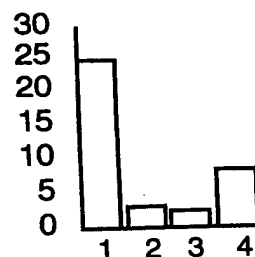
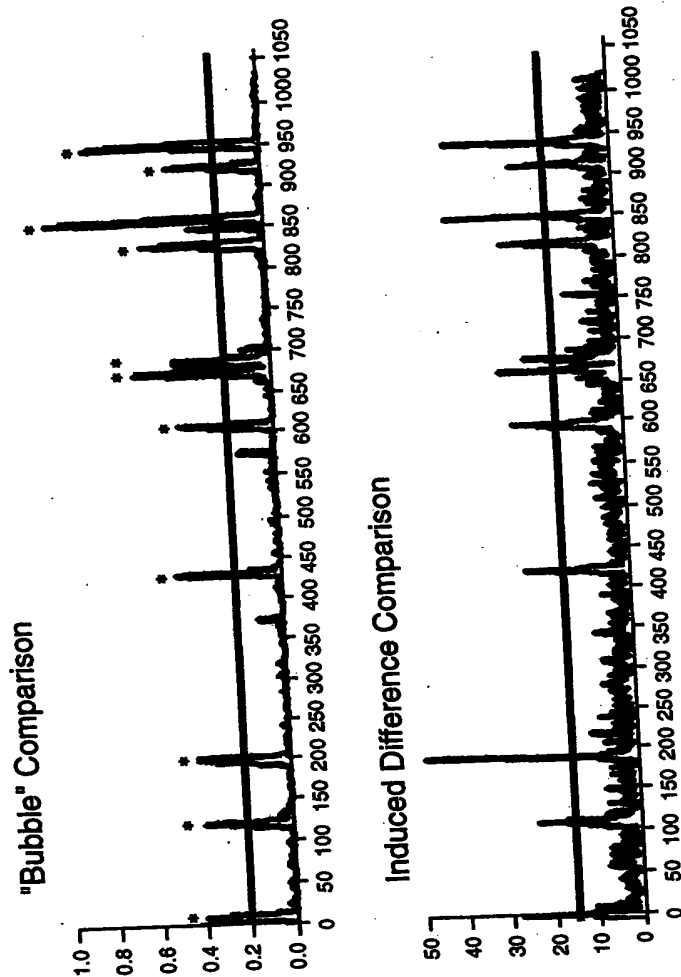


Figure 32b

# Mutation Detection Using Comparisons Between a Reference Target and a Sample Target



Results: No false positives, all 11 mutations (indicated by \*) are detected  
in this 1041 bp HIV DNA fragment.

Figure 33